The Medical Countermeasures Initiative (MCMi) Presents:

2014 MCMi Regulatory Science Symposium

June 2-3, 2014

FDA Headquarters, White Oak Campus
Silver Spring, MD

U.S. Food and Drug Administration
www.fda.gov/medicalcountermeasures
#FDARegSci

June 2, 2014

WELCOME

Dear Colleagues:

Welcome to FDA's third ***MCMi Regulatory Science Symposium***. FDA scientists within the medical product centers, with the support of other offices, including the Office of the Chief Scientist and its Office of Counterterrorism and Emerging Threats, continue to work hard to foster the development and approval of safe and effective medical countermeasures (MCMs).

Since FDA launched our Medical Countermeasures Initiative (MCMi) in 2010, we have dedicated ourselves to strengthening our regulatory science program to facilitate access to safe, effective MCMs. Working with industry and our government colleagues, we have collaborated to further develop and expand availability of medical countermeasures to protect against chemical, biological, radiological, nuclear (CBRN), and emerging infectious disease threats.

You'll hear from many of our partners over the next two days about the exciting—and challenging—work in progress to help support regulatory decision-making through science, and to develop next-generation products to protect against CBRN and emerging naturally occurring threats. With science as the foundation of virtually every FDA regulatory decision, our goal is to continue to support the incorporation of new innovative technologies into the regulatory review process to make product development more efficient and more predictable. We invite you to closely examine some of the research projects currently underway through our poster session, and exchange ideas on MCM development and collaboration with colleagues—one of the main reasons we host this meeting.

Many thanks go to our hard-working staff members who have again worked tirelessly to plan and execute this Symposium. A special thanks to the presenters for their willingness to share their perspectives and for traveling sometimes great distances to contribute their knowledge to the field. Last, but far from least, we want to take this opportunity to again thank all of you for your participation in this event, as well as your innovation, enthusiasm and commitment to this critical national effort.

Sincerely,
Luciana Borio, M.D., and Stephen Ostroff, M.D.

The views expressed in this Regulatory Science Symposium Program and Abstracts Book are those of the authors and do not necessarily reflect the official policy or position of the U.S. Food and Drug Administration, the Department of Health and Human Services, or the United States Government, and should not be used for advertising or product endorsement purposes. Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its approval, endorsement, recommendation, or favoring by the United States Government or any department, agency, office, or branch thereof. The abstracts presented herein have been reproduced as they were submitted by the authors, without editing or amendment of content.

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Moderator: Tom Warf, M.S., Biomedical Advanced Research and Development Authority (BARDA), U.S. Department of Health and Human Services (HHS)

MASSIVELY PARALLEL SEQUENCING FOR MONITORING GENETIC VARIABILITY OF SEED VIRUSES FOR INFLUENZA VACCINE PRODUCTION
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Elizabeth Gonzalez, Ph.D., FDA

10:35 AM BREAK

10:50 AM **MCM IGNITE:** A SERIES OF RAPID, 5 MINUTE PRESENTATIONS ON CURRENT MEDICAL COUNTERMEASURE (MCM)-RELATED ACTIVITIES

11:30 AM LUNCH / POSTER SESSION

1:15 PM **KEYNOTE PRESENTATION**
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Jennifer Reed, Ph.D., FDA

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Venkateswara Simhadri, Ph.D., FDA

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Jane Bai, Ph.D., FDA

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Cristin Welle, Ph.D., FDA

THE PATHOGENESIS AND MANAGEMENT OF ANTHRAX SEPSIS AND SEPTIC SHOCK: STUDIES IN SMALL AND LARGE ANIMAL MODELS
Peter Eichacker, M.D., National Institutes of Health (NIH)

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9:00 AM WELCOME
Margaret Hamburg, M.D., Commissioner, FDA

9:05 AM **KEYNOTE PRESENTATION**
Garry Nolan, Ph.D., Professor of Microbiology and Immunology, Stanford University School of Medicine

10:00 AM **SESSION 3: NEW DIAGNOSTIC STRATEGIES**
Moderator: Randall Kincaid, Ph.D., NIH

NOVEL NANOPARTICLE AND MICROFLUIDICS APPROACHES FOR DETECTION OF VIRAL PATHOGENS IN LABORATORY-BASED AND POINT-OF-CARE SETTINGS
Jiangqin Zhao, M.D., Ph.D., FDA

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Robert Duncan, Ph.D., FDA

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ENABLING SEQUENCE-BASED TECHNOLOGIES FOR MICROBIAL COUNTERMEASURES AND CLINICAL DIAGNOSTICS
Heike Sichtig, Ph.D., and Zivana Tezak, Ph.D., FDA

11:30 AM BREAK

11:45 AM **MCM IGNITE:** A SERIES OF RAPID, 5 MINUTE PRESENTATIONS ON CURRENT MCM-RELATED ACTIVITIES

12:15 PM LUNCH / POSTER SESSION

2:00 PM **PRESENTATION: WHAT'S NEW IN ANIMAL MODEL DEVELOPMENT?**
Andrea Powell, Ph.D., FDA

2:30 PM **PRESENTATION: MOBILIZING THE 2014 FDA-SPONSORED UTMB BSL4 DATA QUALITY COURSE**
Melissa Eitzen, M.S., University of Texas Medical Branch at Galveston

2:45 PM BREAK

3:00 PM **SESSION 4: MCM SURVEILLANCE AND ASSESSMENT**
Moderator: CAPT Carmen Maher, FDA

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Melissa McClung, M.S.P.H., Denver Public Health

REAL-TIME APPLICATION FOR PORTABLE INTERACTIVE DEVICES (RAPID)
Henry Francis, M.D., FDA

4:30 PM CLOSING REMARKS

SPEAKER BIOGRAPHIES

Jane Bai, Ph.D.

Dr. Bai has extensive experience in applying systems pharmacology approaches to integrating pathway analysis, extended pharmacological networks, biochemical homeostasis, and clinical phenotypes of adverse drug reactions for translational assessment of drug toxicity. Her ultimate goal is to achieve systems-pharmacology-guided personalized medicine. Specific examples of her research include non-QT cardiotoxicity of tyrosine kinase inhibitors, drug-induced neuropathy, drug-induced rhabdomyolysis, and hypomagnesemia induced by proton pump inhibitors, and repurposing drugs for treating lung injury induced by toxins. Another effort is analyzing perturbation of biochemical flux and intracellular biochemical homeostasis to understand how gene-drug interactions affect treatment-related clinical phenotypes. Dr. Bai applies her expertise in the safety consult reviews of regulatory submissions and post-approval safety issues, and in conducting cross-office, cross-center, and cross-institute collaborative research activities. She actively engages eminent systems biology and systems pharmacology experts in collaboration to advance regulatory sciences for achieving CDER's and FDA's mission. She has 44 publications in peer-reviewed journals, 3 book chapters and 1 manuscript on systems pharmacology analysis of drug-induced neuropathy accepted for publication in CTP: pharmacometrics and systems pharmacology. Among them, there are approximately 15 papers related to systems pharmacology. Dr. Bai has been invited to guest-edit theme issues including the systems pharmacology theme issue for Biopharmaceutics and Drug Disposition. Dr. Bai is a member of the Steering Committee of NIH LINCS (Library of Integrated Network-based Cellular Signatures) Program, and is an advisor to the Editors of Journal of Pharmaceutical Sciences for her expertise in systems pharmacology.

Paul Buehler, Pharm.D., Ph.D.

Dr. Buehler's laboratory research interests are focused on mechanisms driving the toxicity of extracellular hemoglobin (Hb) in vivo and approaches to minimizing Hb's toxic effects. Intravascular hemolysis caused by genetic, acquired and drug-induced red blood cell (RBC) lysis, as well as stored RBC administration are relevant scenarios in my field of study. Understanding the regulation of endogenous clearance pathways in limiting Hb mediated toxicity is critical to this area of work. Dr. Buehler is currently studying the systems of endogenous Hb clearance as a model to help limit extracellular Hb toxicity either by up-regulation or supplementation of Hb binding proteins (haptoglobins) or macrophage cell surface receptors (CD163). Additionally, his lab is focused on developing pre-clinical animal models of antioxidant depletion, endothelial dysfunction and sepsis along with identification of sensitive biomarkers of oxidative toxicity at the protein, cellular and tissue levels. These models and biomarkers can then be used to evaluate acute and long-term effects associated with both Hb toxicity and detoxification strategies. Taken together, his lab's approaches provide a model system to target therapeutics aimed at minimizing the adverse effects of extracellular Hb as well as to screen and evaluate efficacy, pharmacokinetics and toxicity of potential therapeutic interventions.

Yuansha Chen, Ph.D.

Dr. Chen's expertise is in microbial genomics and antibiotics resistance. Dr. Chen's research supports the regulatory mission of FDA, specifically in the area of monitoring microbial pathogens and their antibiotics resistance in food. As a Research Bioinformatics Analyst, Dr. Chen's major duty is to analyze research data generated by the newest technology applied to pathogen and antibiotics resistance detection. Such technologies include whole genome sequencing, microarray and other high throughput technique. Dr. Chen has reviewed the mechanisms of antibiotics resistance based on published work and summarized this information into a comprehensive database, which was then used to design a microarray for the Medical Countermeasures initiative within FDA and other agencies. Recently, Dr. Chen's team has adopted whole genome sequencing technique in monitoring food borne pathogens and their resistance. In this regard, Dr. Chen has participated in protocol development and evaluation and has set up the bioinformatics pipeline for this technique. Dr. Chen has analyzed data generated by such techniques in order to investigate the phenotypic characteristics and track the spread of pathogens such as *Salmonella*, *Campylobacter sp*., *E. coli* and other organisms of public health concern.

Konstantin Chumakov, Ph.D., D.Sci.

Dr. Chumakov is an Associate Director for Research at the Office of Vaccines Research and Review at the U.S. Food and Drug Administration. He holds a Ph.D. (1979) in molecular virology and Doctor of Sciences degree (1987) from Moscow State University. From 1973-1987 he was a Research Scientist at the Laboratory of Molecular Biology and Bioorganic Chemistry of Moscow State University. From 1987 to 1989, he was a Chief of the Laboratory of Bacterial Genetics at the Institute of Microbiology of the Soviet Academy of Sciences in Moscow. In 1989, he moved to the FDA Center for Biologics Evaluation and Research (CBER) in Bethesda, Maryland, and since 1997 he has served as head of the Laboratory of Method Development in the Division of Viral Products, CBER. His scientific interests are in molecular virology and bioinformatics, creation of molecular methods for evaluation and quality control of vaccines and other biological products as well as development of new approaches for control and elimination of viral diseases. He is an author of more than 150 scientific papers in scientific journals and book chapters.

Robert Duncan, Ph.D.

Dr. Duncan is currently the principal investigator of the research program entitled, "Advanced Technology for Reducing the Risk of Transmission by Transfusion." His expertise in this program is his knowledge of the biology and pathogenesis of blood borne disease organisms including viruses, bacteria and protozoan parasites. He has developed skills in pathogen detection embracing immunological, nucleic acid amplification, real-time PCR, microarray and spectroscopic methods. He has published eight peer-reviewed papers on this topic since 2004. Working closely with biotechnology company scientists who have developed new devices such as nanofluidic real-time PCR plates, resequencing microarrays and laser induced breakdown spectrum analyzers, he has acquired new skills in the application of these devices to multiplex testing of blood for pathogens.

Peter Eichacker, M.D.

Dr. Eichacker is currently a Senior Investigator and Head of the Critical Care Medicine Section in the Clinical Center's Critical Care Medicine Department (CCMD) at the National Institutes of Health. He earned his undergraduate degree from Boston University and his medical degree from New York University. After he completed a residency and chief residency in internal medicine, he had a fellowship in Pulmonary Medicine at Bronx Municipal Hospital Center and Hospital of the Albert Einstein College of Medicine in New York. Dr. Eichacker joined the National Institutes of Health in 1986 as a fellow in critical care medicine. He became a member of the CCMD senior staff in 1991. His primary research interests have been the pathogenesis and treatment of septic shock and sepsis-induced lung injury related to commonly encountered types of bacteria as well as to the less-common anthrax bacterium.

Melissa Eitzen, M.S.

Melissa Eitzen is the Director of Regulatory Operations for the Institutional Office of Regulated Nonclinical Studies (ORNcS) at The University of Texas Medical Branch at Galveston. Regulatory Operations provides independent quality assurance inspections, quality system design, regulatory education, GLP records retention and electronic document management system. Ms. Eitzen works closely with investigators to assure regulatory compliance by implementing quality systems, performing compliance gap analysis, developing risk mitigation strategies to meet compliance requirements, developing quality management plans, and providing GLP compliance education. Ms. Eitzen possesses over thirty years of laboratory development, management, equipment validation, regulatory compliance, laboratory accreditation, and course development experience in medical, environmental, and research laboratories. Her experience in the development of quality systems to meet regulatory requirements began in 1985 when she developed a quality system infrastructure for a new environmental testing laboratory that resulted in dual accreditation by the National Voluntary Laboratory Accreditation Program (NVLAP) and American Industrial Hygiene Association (AIHA). She is currently serving as project director for the Academic Development of a Training Program for GLP in ABSL3/4, a collaborative educational project with the U.S. Food and Drug Administration.

Henry Francis, M.D.

Dr. Francis is the Director of the Data Mining and Informatics Evaluation and Research Group in the Office of Translational Sciences, CDER, FDA. In that capacity, he directs a transdisciplinary group of senior sciences to test, create and operate data analysis program facilitating the efficient use of scientific methods to evaluate complex data information in order to make regulatory decisions for drug approval and drug safety. From October of 2007 until March 2013, Dr. Francis was the deputy director of the Office of Surveillance and Epidemiology (OSE) in the Center for Drug Evaluation and Research (CDER) in the Food and Drug Administration (FDA). Dr. Francis worked with the OSE director to lead 5 divisions of pharmacy and clinical scientists in the detection and study of adverse medical events (AEs) occurring after the release of new drugs into the American health market, also called the post market period. Dr. Francis's specific interest is in the development of data mining techniques to enhance pharmacovigliance capabilities in national medication use and health care databases. Prior to working in FDA, Dr

Francis was a basic and clinical researcher in the National Institute of Allergy and Infectious Diseases in the National Institutes of Health (NIH). As an AIDS clinical investigator, he worked in several clinical and epidemiologic research projects conducting AIDS and tropical research projects in the Democratic Republic of the Congo (DRC, formerly known as Zaire) and other projects in the Caribbean and the South Pacific. In the DRC, Dr. Francis was the Director of the U.S. Public Health Service & Belgian Project SIDA (AIDS research) Research Laboratories in Kinshasa, DRC. Dr. Francis served as the first Director of the National Institute on Drug Abuse's (NIDA) Center on AIDS and Other Medical Consequences of Drug Abuse (CAMCODA). CAMCODA's mission was to establish sustainable AIDS-specific research projects in coordination with the other NIDA projects investigating drug abuse prevention and treatment. As a clinician, Dr. Francis was an assistant professor of medicine at the Johns Hopkins University School of Medicine's Division of Infectious Diseases. He completed his Internal Medicine residency training at the Long Beach VA Hospital in Long Beach, California and his infectious diseases specialty training at the Johns Hopkins Hospital.

Elizabeth Gonzalez, Ph.D.

Dr. Elizabeth Gonzalez is a Staff Fellow (Microbiologist) in the Division of Biology, Office of Science and Engineering Laboratories, CDRH. Dr. Gonzalez received her Ph.D. at the University of Maryland, College Park in Cell Biology and Molecular Genetics in 2012. In August 2012, she started working at CDRH in the Infection Control Lab, which has been focused on how device design influences infection control. The lab has specifically worked on cleaning and disinfecting surrogate select agent pathogens and surrogates of chemical toxins from reusable medical devices and equipment such as pulse oximeters, EKG leads, bed rails, anesthesia machines and ventilators. She has given an oral presentation at the American Society for Microbiology Biodefense meeting and has presented the data in multiple posters at FDA MCMi Symposia and ASM Biodefense meetings. In addition, she has published several papers in peer reviewed journals.

Heather Hawley, B.A., B.S.

Heather Hawley is the Senior Principal Program Manager for the Food and Drug Administration at the MITRE Corporation. Ms. Hawley leads teams across the FDA, focusing on fulfilling the potential of Information Technology and Informatics as a mission enabler and force multiplier. Heather brings more than two decades of professional experience in large-scale systems and software design, development, and implementation in federal government and commercial sectors. Heather has leveraged her extensive data analytics and business intelligence experience on behalf of a wide range of customers in a variety of industries including the U.S. Food and Drug Administration, the Internal Revenue Service (IRS), U.S. Securities and Exchange Commission, National Aeronautical and Space Administration, telecommunications carriers and local exchanges, and commercial banking. Heather holds two bachelor's degrees from the University of Maryland (B.S. in Computer Science, with a minor in Mathematics and a B.A. in Psychology, with a minor in French).

Mansoor Khan, Ph.D.

Dr. Mansoor A. Khan is the Director of Product Quality Research at CDER in FDA. Prior to joining FDA in 2004, Dr. Khan was a Professor of Pharmaceutics and Director of Graduate Program in the School of Pharmacy at Texas Tech University. He has published over 240 peer-reviewed manuscripts on the manufacturing sciences and bioavailability/bioequivalence issues of drug products, four texts including "Pharmaceutical and Clinical Calculations," 20 book chapters, 200 abstracts/poster presentations, and more than 175 invited presentations worldwide. He has held several leadership positions at the American Association of Pharmaceutical Scientists (AAPS) including elected chair of pharmaceutics and drug delivery (PDD) and the founding chair of formulations design and development (FDD). Dr. Khan recently received the 2012 AAPS Research Achievement Award in Formulations Design and Development. He is also an AAPS Fellow. Dr. Khan serves on the editorial board of Pharmaceutical Technology, International Journal of Pharmaceutics, AAPSPharmsciTech, Drug Delivery and Translational Research, and the Journal of Clinical Research and Regulatory Affairs.

Melissa McClung, M.S.P.H.

Melissa McClung has managed the Hand-held Automated Notification for Drugs and Immunization (HANDI) project at Denver Public Health for the past four years. HANDI is a mobile app created as a data collection tool to support mass intervention events. Involved since HANDI's inception, her responsibilities have included requirements and use case development, vendor collaboration, testing, implementation and application support during HANDI's use at immunization clinics and emergency preparedness exercises. At Denver Public Health for 14 years, Melissa's previous work experience includes developing a syndromic surveillance system at the Denver Center for Public Health Preparedness, providing data and GIS analysis for a CDC-funded motor vehicle crash injury prevention grant, and software development for several software consulting firms. She holds a Bachelor of Science degree in Applied Mathematics with an emphasis in Computer Science from the University of Colorado, Boulder and received her Master of Science degree in Public Health from the University of Colorado, Denver.

Garry Nolan, Ph.D.

Dr. Nolan is a Professor in Microbiology and Immunology at the Stanford University School of Medicine. His areas of expertise include signaling biology, immunology, cancer biology, retroviral design, bioinformatics, and genetics. His laboratory's recent interests include development of novel methods for detection of RNA targets using branch chain DNA (bDNA), studying signaling alterations at the single cell level in leukemia and lymphomas, cancer stem cells, and determining which of these signaling attributes correlate with patient outcome, drug reactivity, and mechanism of disease progressions. Similarly, his lab works on global representations of signaling attributes and capabilities in the immune system in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. His lab is developing an encompassing reference of the human immune system in terms of its variation in response to biothreat agents, countermeasures and biological stimuli across age, gender and ethnicity

and in terms of its differences compared to the common model organisms. His lab also develops and applies advanced tools in informatics, such as machine learning, as implemented specifically for single cell analysis and use of perturbation analysis. In addition, his lab also has an extensive program in the development of hardware algorithm implementations (using both field programmable gate arrays as well as graphical processing units) for highly parallelized machine learning of network structures.

Andrea Powell, Ph.D.

Andrea Powell received her doctorate in pharmacology from Cornell University Graduate School of Medical Sciences. After 21 years of regulatory experience in FDA's Center for Drug Evaluation and Research (CDER) as a pharmacology/toxicology reviewer of drugs to treat neurological conditions, she joined CDER's Office of Counter-Terrorism and Emergency Coordination (OCTEC) in 2008 to facilitate the development of medical countermeasures for chemical, biological, radiological, and nuclear agents. As part of OCTEC's Medical Countermeasures team, she works on policy and guidances related to product development under the Animal Rule, and on the development of electronic data standards for Animal Rule-specific studies.

Jennifer Reed, Ph.D.

Dr. Reed has approximately 20 years of experience in development of accessible animal models that reflect mechanisms of severe disease pathogenesis. Her research emphasis has been the understanding of susceptibility to severe outcomes after viral infection. Dr. Reed's recent studies toward development of an animal model of eczema vaccinatum (EV), a severe disseminated infection that occurs in some persons with atopic dermatitis (AD) or other skin disorders, have uncovered novel components of early antiviral responses in the skin. AD affects approximately 10% of the U.S. population. Should the U.S. return to universal vaccination, thousands of EV cases would be expected, with prolonged hospitalization and a fatality rate of up to 40% in untreated patients. The only licensed treatment for EV, vaccinia immune globulin (VIG), has not been systematically studied in a controlled clinical trial. The understanding of VIG mechanism of action is very limited. There is currently no animal model of EV in which optimal dosing and/or co-therapies can be evaluated. Dr. Reed's work may inform the therapeutic use of VIG, and streamline approval of dose-sparing co-therapies. Dr. Reed received a Ph.D. from The Johns Hopkins University.

Jo Ellen Schweinle, M.D.

Dr. Schweinle is the director of the Division of Clinical Studies at the Biomedical Advanced Research and Development Authority (BARDA). Before joining BARDA Dr. Schweinle was Vice President of Medical Affairs at Axcan Pharma, a specialty pharmaceutical company. She has also worked for Bayer Healthcare Pharmaceuticals, GlaxoSmithKline, McNeil Consumer and Specialty Pharmaceuticals (Johnson & Johnson), Chiron Pharma, and Novartis as Director/Senior

Director of Clinical Development for Medical Affairs. She has been primarily responsible for clinical development of anti-bacterial and anti-viral agents but has also been responsible for cardiovascular, central nervous system, pulmonary, gastrointestinal, metabolic, urinary tract, and arthritis/pain medications. She has led "lifecycle management" teams for multiple products and worked on the transitions of a prescription drug and a device to over-the-counter availability. While on the faculty at the University of Texas Medical Branch (UTMB) and at Yale Medical School, her research was supported by two NIH grants and other grants. She was a visiting scientist in the National Institute of Allergy and Infectious Diseases at NIH during which time she investigated complement-mediated immunity and microbial pathogenesis. Dr. Schweinle has authored numerous peer-reviewed publications and book chapters and was twice awarded the Harriet Cunningham Citation for Meritorious Scientific Writing. Dr. Schweinle received an MD from UTMB and a BS in Psychology from Sophia University in Tokyo, Japan. She is board certified in the specialty of Internal Medicine and the sub specialty of Infectious Diseases.

Heike Sichtig, Ph.D.

Dr. Sichtig is a subject matter expert and premarket reviewer within the Division of Microbiology Devices in the Office of In -vitro Diagnostics and Radiological Health (OIR), Center for Devices and Radiological Health (CDRH) at the U.S. Food and Drug Administration. She is the lead technical regulatory scientist for microbial diagnostic devices related to high throughput sequencing and bioinformatics. She is developing approaches to use alternative analytical models for assessing safety and effectiveness for these novel sequence-based diagnostic devices. She has significant experience developing methods and computational tools for bioinformatics with the biologist in mind. Prior to joining the FDA, Dr. Sichtig developed and assessed a novel adaptive computational modeling platform for transcription factor binding site detection and discovery using machine learning techniques (artificial spiking neural networks and genetic algorithms) at the Department of Molecular Genetics and Microbiology at University of Florida, Gainesville, Florida. She received her Ph.D. (2009) in Biomedical Engineering from Binghamton University, Binghamton, New York, her M.Sc. (2004) in Computing, Statistics and Math and her B. Sc. (2003) in Computer Science from Kean University, Union, NJ. She has experience in teaching courses such as Bioinformatics, Complex Biological Systems, Autonomous Agents, Probabilistic Systems and Biological Networks. She is Part-Editor for the Handbook of Bio – and Neuroinformatics and is on the Advisory Board for the Springer Series in Bio-Neuroinformatics. She is a member of the CLSI Consensus Committee on Molecular Methods and many professional societies including IEEE CIS, INNS, ASM and ASEE. She is the current chair of the IEEE CIS Chapters Sub-Committee and is a member of the Bioinformatics and Bioengineering Technical Committee. Her diverse background spanning from computer science and engineering to molecular biology and bioinformatics requires superior communication skills to navigate between research fields.

Venkateswara Simhadri, Ph.D.

Dr. Simhadri's previous and current research involves multiple scientific areas. Dr. Simhadri's initial research experience as a Junior Research Fellow in molecular biology and biochemistry set a platform for Ph.D. studies. During Dr. Simhadri's Ph.D. and post-doctoral research programs, Dr. Simhadri focused on the field of immunology and continued to work on aspects of natural immunity. Dr. Simhadri's main focus was to identify the ligands for different receptors in the innate immune system and to delineate the functional roles in the context of receptor-ligand interactions.

Zivana Težak, Ph.D.

Dr. Težak is an Associate Director for Science and Technology, Personalized Medicine Staff, in the Office of In Vitro Diagnostic Device (IVD) Evaluation and Safety (OIR), at the Center for Devices and Radiological Health, Food and Drug Administration (FDA). Prior to joining the FDA in 2004 as a scientific reviewer in microbiology, genomics and molecular biology, Dr. Težak worked in the biotechnology industry, holding research and development scientist positions in a bioinformatics and array development company. Dr. Težak received a Ph.D. in Biochemistry/Molecular Biology from Florida State University in 1997. From 1998 to 2001 she was a research fellow at the University of Pittsburgh Medical Center and Children's National Medical Center, Research Center for Genetic Medicine, working on neuromuscular disorders, human genetics, gene therapy, and high-throughput screening technologies. Her work resulted in a number of publications in peer-reviewed journals, book chapters and proceedings. In her current position, Dr. Težak has been leading efforts to develop flexible regulatory policies for novel technology based IVDs, such as next-generation sequencing, in order to enable their smoother translation into the clinic.

Cristin Welle, Ph.D.

As the principal investigator of the Neural Implant Lab in the Division of Physics at FDA, Dr. Welle directs a team of scientists in the development of test platforms to evaluate the long-term safety and reliability of neural interface devices in small animal model systems. The lab is currently investigating invasive neural recording electrodes used in neuroprosthetic systems, and novel electrode technology and qEEG endpoints for the detection of traumatic brain injury. The lab's goal is to contribute to the scientific knowledge base required to speed innovative neural device development and regulatory review. Dr. Welle brings expertise in neurophysiology and cortical microcircuitry from her graduate and postdoctoral experience at the University of Pennsylvania. The Neural Implant Lab receives funding from the DARPA RE-NET Program and the FDA's Medical Countermeasure and Critical Path Initiatives and collaborates with government, academic and industry researchers. In addition to these research efforts, Dr. Welle also provides subject matter expert consulting reviews for neurological device submissions to the Office of Device Evaluation. She participates in internal and external efforts to facilitate the regulatory process for neural technology, including serving as co-chair of the Clinical BCI working group, a team member of the FDA's Innovation Pathway Pilot (1.0), and SME for DARPA, NIH and VA neurotechnology grant review.

Janet Woodcock, M.D.

Dr. Woodcock joined FDA in 1986, assuming the leadership of the Center for Drug Evaluation and Research (CDER) in May 1994. Prior to joining CDER, she served as Acting Deputy Center Director of the Center for Biologics Evaluation and Research (1990 -1992) and Director of the Office of Therapeutics Research and Review (1992 - 1994), where she oversaw approval of the first biotechnology-based treatments for multiple sclerosis and cystic fibrosis. From 2004 to 2008, Dr. Woodcock provided support to FDA's Commissioner, serving as Deputy Commissioner and Chief Medical Officer, Deputy Commissioner for Operations and Chief Operating Officer, overseeing various aspects of scientific and regulatory operations. During her tenure at FDA, Dr. Woodcock's achievements have been substantial. Under her leadership, CDER has streamlined review processes for new and generic drugs while improving standards for quality, safety, and effectiveness. The processes for submitting marketing applications and adverse events reports and for reviewing submissions in FDA have been automated. CDER's regulatory decision-making processes also have been streamlined, making decisions more open and transparent. CDER's regulatory procedures and policies are publicly available — scores of technical guidances describing FDA's thinking on regulatory standards have been issued. Many CDER process are carried out with an unprecedented degree of participation on the part of consumer and patient representatives. An extensive CDER Web site hosts a myriad of helpful information on drug approvals, safety issues, and other critical information targeting consumers, patients, health care practitioners, regulated industry, and other audiences. In 2011 and 2012, Dr. Woodcock launched multiple efforts to support development of new therapies for rare and neglected diseases, molecularly defined disease subgroups, and new antibacterial therapies. She oversaw the implementation of innovative policies to foster adaptive trial designs (2010) and trial enrichment strategies (2012) and encourage the qualification of new drug development tools (2010) to help speed drug development and evaluation. Following enactment in March 2010 of the Patient Protection and Affordable Care Act (Affordable Care Act), Dr. Woodcock developed and launched the biosimilars effort to create an abbreviated licensure pathway for biological products; she then negotiated the Biosimilar User Fee Act of 2012 (BsUFA) to support approval using this new pathway. Dr. Woodcock continues to lead FDA's Pharmaceutical Quality for the 21st Century initiative to modernize pharmaceutical manufacturing and the Safe Use/Safety First initiatives, which are critical to drug safety throughout the drug lifecycle and ensuring frequent and clear communications to the public about the risks and benefits of drugs. As Director of CDER, Dr. Woodcock maintains contact with a variety of diverse constituencies, including the clinical and scientific communities, members of Congress and the Administration, patient and consumer advocacy groups, the international drug regulatory community, regulated industry, and representatives of Federal and State agencies. She frequently appears in or is quoted by the national media and has testified repeatedly before Congress. Dr. Woodcock has earned numerous awards, most recently, the Arthritis Foundation's Floyd B. Odlum Making a Difference Award and the Luminary Award from the Personalized Medicine World Conference. She has been the recipient of the Presidential Rank Meritorious Executive Award and three HHS Secretary's Distinguished Service Awards among many others. She has authored more than 60 publications.

Jiangqin Zhao, M.D., Ph.D.

Dr. Zhao has many years of experience in molecular biology, virology and infectious disease research, particularly HIV and working on biodefense agents including influenza and anthrax. Dr. Zhao has been involved in developing diagnostics for influenza and various biodefense pathogens based on microarray and nanoarray/fluidics technologies. Sensitive antigen assays and genomic arrays have been developed to detect multiple strains of influenza. Dr. Zhao has also recently introduced high throughput deep sequencing to characterize multiple influenza strains in patient samples, while conducting studies to determine the tropism of influenza viruses for various types of blood cells since viremia has been observed in acute influenza infection.

POSTER SESSION

Animal Models

Poster No.

1.1 **McFarland, Hugh**
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POSTER ABSTRACTS

Animal Models

1.1 Rescue of Vaccine T-cell Memory Following Sublethal γ Irradiation

McFarland, Hugh; Berkson, Julia; Lee, Jay; Xie, Hang; Elkahloun, Abdel; Rosenberg, Amy. *U.S. Food and Drug Administration*

Background: Subsequent to sublethal γ radiation exposure, survivors reacquire immune competence in the following weeks, but lose vaccine immunity with loss of memory T cells. Our goal is to "rescue" memory T cells from apoptotic death to improve disease resistance following γ radiation exposure.

Methods: Mice were immunized with attenuated Listeria monocytogenes, exposed to 6 Gy radiation, and revaccinated. Mice were either challenged with wild-type Listeria, or spleens were harvested for quantitative PCR and cDNA array analysis 6 hrs. following rescue.

Results: We have found that rapid revaccination of Listeria (LM) immunized mice following irradiation preserves immunity. The "window" of time in which rescue is effective is 0-3 days following radiation exposure, and gradually declines thereafter. Mice are able to survive infection with the vaccine strain LM-ActA only when given during the window of rescue with increasing lethality thereafter. Animals surviving revaccination can survive a lethal challenge with the wild type strain of LM 4 weeks later, demonstrating preservation of immunity. A group of cytokines and chemokines have been identified by cDNA arrays and qPCR that are uniquely up-regulated in the spleens of D1 but not D4 post-irradiation (PI) LM-rescued animals: IRG1, IL-36γ, CXCL9, CXCL10, interferon γ, IL-15 and IL-2 among others. Myeloid cells are the likely source of some of many of these products as they were produced by the macrophage cell line RAW264.7 following irradiation and rescue. Other factors such as IFNγ and IL-2 are T cell products and D1-rescued CD8+ T cells up-regulate IFNγ and IRG1.

Conclusion: We show for the first time that bacterial infection and γ radiation synergize in the generation of chemokines, cytokines, and other genes. We hope to use this information to find alternatives to revaccination rescue of immunity that can be stockpiled and easily administered to victims of ionizing radiation exposure.

1.2 Raxibacumab Treatment Improves Survival in a Fluid and Vasopressor Supported Canine Model of B. anthracis Lethal and Edema Toxin Associated Shock

Remy, Kenneth E.[1]; Cui, Xizhong[1]; Solomon, Steven D.[1]; Sun, Junfeng[1]; Li, Yan[1]; Fitz, Yvonne[1]; Eichacker, Peter Q.[1].

[1]*National Institutes of Health; Clinical Center, Department of Critical Care Medicine, Bethesda, MD*

Background: Shock in patients with *B. anthracis* infection appears particularly resistant to conventional hemodynamic support with fluid and vasopressors. In a review of 27 confirmed cases from the 2009 UK outbreak, eleven of fourteen patients requiring vasopressor treatment at any time during hospitalization died. Based on evidence that therapies inhibiting *B. anthracis* lethal (LT) and edema (ET) toxin improve outcome during infection, two toxin directed agents, anthrax immune globulin (a polyclonal antibody) and raxibacumab (RAXI, a monoclonal antibody directed against the protective antigen component of both LT and ET) have been included in the Strategic National Stockpile. However, it is unknown whether either agent is beneficial when combined with conventional hemodynamic support in subjects developing shock related to LT and ET release. Since LT and ET are released in combination during infection, we investigated the effects of RAXI in canines challenged with 24h infusions of LT and ET together. The HS employed was shown previously to improve outcome during LT or ET challenges alone.

Methods: Sedated, mechanically ventilated canines received 24h infusions of LT and ET combined in equal molar doses. At the start of toxin infusion, animals were randomized to HS alone (normal saline and norepinephrine titrated based on systemic and pulmonary artery pressures respectively throughout study, n=5), HS combined with a single dose of RAXI administered at the start of toxin (HS+T0mAb, n=4), or 6h afterwards (HS+T6mAb, n=3). Hemodynamic changes were monitored continuously and animals were observed for 96 h and then euthanized. A daily shock score accounting for both the average mean arterial blood pressure (MBP) and amount of norepinephrine animals had received over the prior 24 h period was calculated (decreased score represents decreased MBP and increased norepinephrine requirement).

Results: While the mean shock score (Figure 1) began to decrease by 60h in animals receiving HS only, it did not decrease in HS+T0mAb and HS+T6mAb animals and remained unchanged throughout. All five animals receiving HS alone died (Figure 2) before 96 h [individual survival times 65, 71, 71.5, 86 and 92h and mean survival 77.8±4.4] while the four HS+T0mAb animals and the three HS+T6mAb animals all survived until the end of the 96h observation period ($p=0.03$ comparing HS alone versus HS+mAb at T0 and T6 combined).

Conclusion: These results suggest that RAXI in combination with HS has benefit compared to HS alone during shock related to LT and ET together in this canine model. Further defining the clinical relevance of these results is necessary.

1.3 Recombinant Protective Antigen Vaccine (SparVax®) Provides Protection Equivalent to Biothrax® against Lethal Challenge with Bacillus anthracis in New Zealand White Rabbits

Crowe, Sherry; Nelson III, Otis; Fusco, Peter. *PharmAthene, Inc.*

Background: Extensive efforts have been directed toward development of subunit vaccines based on recombinant protective antigen (rPA), the principal virulence factor of *Bacillus anthracis*. PharmAthene is developing an rPA vaccine adsorbed on Alhydrogel® (SparVax®) for immunization against inhalational anthrax. Using the New Zealand White rabbit model, a study was designed to compare efficacy and immunogenicity of the rPA vaccine to the currently licensed anthrax vaccine (AVA, Biothrax®).

Methods: Groups of twenty rabbits were vaccinated intramuscularly on days 0 and 28 at target doses of 27, 9 or 3µg rPA. The 9 µg dose of SparVax® was bracketed as this is the proposed human equivalent

dose (HED). Twenty animals received the AVA HED, diluted 1:16 in saline. A control group of 6 rabbits received sterile saline. Rabbits were bled prior to the initial dose and on days 35, 42, 56, and 69 to assay for functional antibody by a toxin neutralization assay (TNA). Animals were challenged with *B. anthracis* Ames strain spores on day 70 via nose-only inhalation.

Results: Immunization with 27 or 9µg rPA resulted in 100% survival, as seen with the AVA immunized group; there was 95% survival in the 3µg rPA group. All control animals succumbed to the infection. The preliminary TNA data show a clear dose response curve for SparVax®, and the ED_{50} and NF_{50} values are consistently higher in the 9 µg dose group than the AVA group. ED_{50} values at days 35, 42, 56, and 69 were 3875, 2408, 1010, and 617, respectively for the 9 µg dose group, compared to 2608, 1695, 609, and 330 for the AVA group. The day 69 NF_{50} values were 1.2 and 0.6 for 9µg rPA and AVA, respectively.

Conclusions: SparVax® is at least equivalent to Biothrax® as measured by either survival or TNA using the rabbit model.

1.4 A Conscious Sheep Model as a Development Tool for Blood Loss Monitors: Characterizing the Hemodynamic Response to Hemorrhage

Scully, Christopher; Kramer, George; Strauss, David. *Division of Physics, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, U.S. Food and Drug Administration*

Background: Traditional patient triage and monitoring during mass casualty incidents consists of patient examination and vital sign "snap-shots." However, because of physiological compensatory mechanisms vital signs can be late indicators of blood loss and may not provide notification until life-saving interventions are necessary. Treatment then requires significantly more resources that are scarce during an initial incident response or that may be unavailable in the field. We investigated an experimental conscious sheep hemorrhage model as a development tool for blood loss monitors.

Methods: Adult sheep (7) underwent one, two or three 25 ml/kg 15 min hemorrhages on separate days (N=14 total experiments). Continuous physiological monitoring included electrocardiogram, arterial blood pressure, respiration belt, and pulmonary artery blood flow (cardiac output). Physiological measurements were compared during baseline, peak heart rate, and post-hemorrhage time points.

Results: For all animals, heart rate, 89±23 BPM (mean ± SD) at baseline, increased during hemorrhage reaching a peak of 169±28 BPM at a median time of 10 min (range: 5-22 min) before retreating to 108±20 BPM post-hemorrhage. Systolic blood pressure decreased from 123±15 to 85±15 to 68±16 mmHg (baseline, peak heart rate, post-hemorrhage), and cardiac output decreased from 4.6±0.8 to 3.6±0.7 to 2.5±0.7 L/min. The breathing rate remained unchanged during hemorrhage (36±14 to 42±24 breaths/min at baseline and peak heart rate) but increased after the hemorrhage to 84±45 breaths/min.

Conclusion: The hemodynamics of conscious sheep showed the traditional mammalian response to hemorrhage. Monitoring variables related to blood loss is a needed, practical and plausible application of new continuous physiological monitoring devices to provide earlier notification of significant hemorrhage and improve patient triage. Large animal models enable studies to test the effect of injury variability on novel blood loss monitoring approaches as well as to compare non-invasive and invasive measures.

1.5 Pharmacokinetics of Oseltamivir During Pregnancy

Lumen, Annie; Loukotková, Lucie; Basavarajappa, Arjun; Chen, James; Fisher, Jeffrey; Gamboa da Costa, Gonçalo; Morris, Suzanne; Paule, Merle; Roberts, Rosemary; Slikker, William; Williams, Denita; Yang, Xiaoxia. *U.S. Food and Drug Administration*

Background: Pregnant women constitute an “at-risk” population in the event of an influenza pandemic. Significant physiological changes occur during pregnancy that alter the pharmacokinetics of drugs, such as oseltamivir (Tamiflu), possibly resulting in `under-dosing'.

Method: A sensitive analytical method was developed to measure oseltamivir and its active metabolite oseltamivir carboxylate in serum. This method will be used in a non-human primate model (NHP) to evaluate the pharmacokinetics of oseltamivir during each trimester of pregnancy in comparison with the non-pregnant conditions.

Results: Preliminary pharmacokinetic studies were conducted in female rhesus monkeys (n=2) to confirm the detection of oseltamivir and oseltamivir carboxylate in the serum and to validate analytical methods. A single dose (1.25 mg/kg) of oseltamivir was administered via intravenous (i.v.) or orally. Analysis of the samples helped optimize the dose and blood sampling times. In another study, six non-pregnant rhesus monkeys were administered 2.5 mg/kg oseltamivir by i.v. (single dosing) or orally (multiple dosing). Following a single i.v. dose, the time to reach maximum serum concentration for oseltamivir carboxylate (529.6±74 ng/ml), ranged from 0.5 to 1 h. Following twice-a-day oral administration for 5 days, the first dose peak concentration and time to peak for the oseltamivir (54.5±31.5 ng/ml) and oseltamivir carboxylate (589.2±130 ng/ml), were 1.0 and 2.0 h, respectively. Results from this pilot study have been used to establish the design of an ongoing study in NHP evaluating the pharmacokinetics through gestation in comparison to pre-pregnancy conditions.

Conclusions: A physiologically based pharmacokinetic model will be developed for the non-pregnant and pregnant NHP and will be used to extrapolate to human pregnancy, where much less pharmacokinetic data are available. The goals of the project are to determine the utility of using NHP to understand better drug dosimetry during human pregnancy and provide insights into the concern of oseltamivir under-dosing during pregnancy.

1.6 Development of a Mamu-A-01 transgenic model to assess Ebola vaccine responses

Wood, Steven; Dutta, Debargh. *U.S. Food and Drug Administration.*

Background: Ebola induces a deadly hemorrhagic fever. Immunity to FV requires both cell-mediated and humoral immunity. Importantly, the surface glycoprotein, GP12Fc, confers protections against Ebola in mice. Rhesus macaques are used to assess Ebola vaccine responses. Recently, a murine transgenic model that expresses Rhesus macaque MHC, Mamu A-01, has been developed.

Study Rationale: Given that the pivotal clinical trials will take place in monkeys, development of assays to follow both cell-mediated and humoral immunity are needed. The Mamu-A-01 transgenic model

provides a means to rapidly screen vaccine candidates. GP12Fc has been shown to confer protection against mouse adapted Ebola virus.

Methods: A GP12 Zaire 15 mer/5 mer overlap mimeotope library was synthesized. Nine pools of 15 overlapping peptides were prepared. Flow cytometry was used to evaluate g-interferon and TNF-a synthesis using intracellular staining (ICS). GP12 Ebola Zaire sequence were analyzed by the Immune Epitope Database (IEDB) to identify immunodominant epitopes. Peptides were synthesized and screened using MHC stability assays. Two Tetramers were prepared by NIAID.

Results: Mice were immunized with GP12Fc and the ICS response in splenocytes was assessed. Interestingly, the first pool induced the most robust response. In silico and MHC stability studies identified two immunodominant epitopes, NTPVYKLDI, AA 390-398 (P20) and FTPQFLLQL, AA 248-256 (P21). Importantly, antigen-specific T cells from GP 12 Fc immunized mice were clearly demonstrated using P20 and P21 Tetramers.

Conclusions: Mamu-A01 mice have been generated and were immunized with the vaccine candidate GP12Fc. ICS revealed a robust response to GP12Fc. Further, IEDB analysis and MHC stability studies identified immunodominant peptides, P20 and P21. Most importantly, tetramers against Ebola have been developed for Mamu-A01 and can detect GP12Fc specific immune cells. Future experiments will focus upon fully cataloging the immune response to vaccine candidates in transgenic mice that express Rhesus macaque Mamu-A01.

1.7 Mitigating long-term cardiac adverse effects of radiation exposure: emerging opportunities in protein oxidation and autophagy modulation

Rosen, Elliot; Gonzalez, Yanira; Aryal, Baikuntha; Chehab, Leena; Dickey, Jennifer; Rao, Ashutosh. *U.S. Food and Drug Administration.*

Radiation-induced heart disease (RIHD) presents a significant challenge in the event of an accidental radiation exposure of a population to ionizing radiation. Likewise, in the oncology setting, patients who receive acute doses of irradiation to the thoracic cavity for Hodgkin's Lymphoma and breast cancer are subject to an elevated risk of RIHD. The ability to mitigate the long-term cardiac effects of radiation exposure in these populations by modulating protein oxidation and the process of autophagy in the heart has the potential to lead to improved health outcomes. We utilized the spontaneously hypertensive rat (SHR), which has proven a good model for drug-induced cardiotoxicity, to evaluate radiation-induced heart disease. SHRs were exposed to low, medium, or high doses of full-body irradiation. Necropsy was performed to collect serum and tissue samples at various time points up to one year. Echocardiography and electrocardiography were performed to evaluate functional changes in cardiac performance. Four weeks following ionizing radiation, both males and females showed decreased heart mass and decreased whole body mass in the groups exposed to medium and high doses of radiation. A marked difference in both heart and whole body mass was observed in the high dose group after one year. Anemic conditions were noted by low red blood cell counts observed in both males and females at all three time points in the animals receiving the highest dose of irradiation. Cardiac troponin T analysis revealed cardiotoxicity in both the medium and high dose groups at earlier time points. Echocardiography revealed that left ventricular volume at diastole was increased in males following high dose irradiation, and that left ventricular mass decreased following high irradiation

exposures. Finally, it should also be noted that after one year, multiple animals receiving radiation developed spontaneous tumors. We are currently exploring the oxidative stress-mediated molecular mechanisms behind the radiation-induced cardiac damage.

1.8 Estimating the Efficacious Human Dose under the Animal Rule for AVI-7288, a Countermeasure Directed against Marburg Virus

Charleston, JS; Warren, T; Iversen, P; Bavari, S; Saoud, J; Heald, A; Kaye, EM; Wong, M; Berry, D; Sazani, P. *Sarepta Therapeutics, Inc.*

Background: Sarepta Therapeutics is developing AVI-7288 under the Animal Rule as a countermeasure against Marburg virus. AVI-7288 dosed daily at 15 mg/kg for 14 days has proven effective in a non-human primate (NHP) Marburg lethal challenge model. Comparison of the pharmacokinetic (PK) exposure profiles associated with this survival benefit with PK profiles in healthy NHPs and healthy human volunteers provides a mechanism to estimate a protective dose in humans and satisfy a critical element of the Animal Rule.

Methods: A pharmacokinetic/pharmacodynamic study of AVI-7288 administered to Marburg virus infected NHPs was conducted to determine the PK exposure profile associated with a survival benefit. A similar study was conducted in uninfected animals to determine the impact of the disease condition on PK profiles. PK profiles were also determined in a Multiple Ascending Dose (MAD) clinical trial in healthy human volunteers receiving daily doses of AVI-7288 at 1, 4, 8, 12 and 16 mg/kg for 14 days.

Results: The 15 mg/kg AVI-7288 AUC_{0-24} exposure of 79,000 hr*ng/mL for 14 days provided survival benefit in the NHP lethal challenge model. This AUC_{0-24} profile was similar in infected and uninfected animals, providing support to compare these PK profiles with those from the MAD clinical trial to estimate a potential efficacious human dose. Modeling this comparison indicates that the protective exposure is achieved following 14 days of dosing in the 8 mg/kg clinical cohort. All doses in the clinical trial were well-tolerated, demonstrating a favorable safety profile.

Conclusion: An AUC_{0-24} of 79,000 hr*ng/mL for 14 days provide 83-100% survival benefit in the NHP lethal challenge model. A dose of 8 mg/kg/day for 14 days exceeds this exposure level in healthy human volunteers. This predicted dose is expected to be protective for human clinical use.

1.9 Application of bioimaging to evaluate the effects of antiviral therapies in mice challenged with vaccinia virus intranasally and via scarification as a surrogate model of progressive vaccinia

Zaitseva, Marina; McCullough, Kevin; Cruz, Stephanie; Golding, Hana. *U.S. Food and Drug Administration.*

Background: Military recruits are routinely vaccinated with live attenuated smallpox vaccine. Complications of vaccination including progressive vaccinia (PV) were reported in individuals with immunodeficiency, eczema, or atopic dermatitis. Therefore, antivirals are essential as a counter-terrorism measure and for treatments of post-vaccination complications. The goal of our project was to

develop animal models including mouse model of PV for evaluation of antiviral drugs and immunotherapies.

Methods: Whole body bioluminescence imaging (WBI) is used to follow in-host dissemination of recombinant vaccinia viruses expressing luciferase enzyme (WRvFire or IHD-J-Luc) in mice after intranasal challenge (IN model) or scarification (PV model). Bioluminescence signals from infected organs are registered by the charge couple device camera in IVIS instrument (PerkinElmer) and are converted into numerical values using Living Image software. We already demonstrated that WBI provides a quantitative approach to evaluate the effect of anti-smallpox therapies including VIGIV, Cidofovir, and ST-246 on viral dissemination to internal organs (lungs, spleen, and liver) and on pox development. In the past year we studied the effect of CMX001 (Chimerix), a biodegradable and safe analogue of Cidofovir.

Results: Three doses of CMX001 starting from day 1 or day 2 post-challenge significantly reduced virus replication in internal organs, protected Balb/C mice from lethality, and reduced pox formation even after the drug was discontinued. Importantly, mice that were protected by CMX001 from lethal challenge with IHD-J survived re-challenge with the same virus and with WRvFire. CMX001 also protected immune deficient (nude) mice from lethality but once the drug was removed, mice succumbed to death. Nude mice partially reconstituted with T cells and treated with CMX001 survived challenge suggesting that CMX001 controlled viral replication for sufficient time allowing viral-specific immune responses to clear the infection.

Conclusions: WBI is very useful for following vaccinia dissemination in IN and PV mouse models and for predicting the effectiveness of anti-viral drugs, VIG, and MAbs.

1.10 The human antibody response to 2012/13 trivalent inactivated influenza vaccine in BLT humanized mice

Fantoni, Giovanna; Abbasi, Fatima; Bauer, Steven; Couzens, Laura; Shirin Treadwell, Patnaik, Anuja; Ragheb, Jack A. and Eichelberger, Maryna C. *Division of Viral Products, Office of Vaccine Research and Review, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration.*

Vaccines are the most effective option for the control and prevention of influenza infection. Preclinical immunogenicity studies of influenza vaccines conducted in mice and ferrets sometimes do not accurately reflect the human immune response. There is a need for an animal model that is more predictive of immunogenicity in humans. We generated BLT humanized mice by engrafting NOD *scid* IL-2 receptor gamma chain knockout (NSG) mice with human thymus and autologous hematopoietic stem cells from 2 different donors (groups I and II) and monitored development of human leukocytes by immunostaining with antibodies specific for human CD45. At 6-7 months post stem cell transfer, >50% of peripheral blood mononuclear cells (PBMC) from group I and 7-10% of PBMC from group II, were of human origin. Mice were vaccinated intramuscularly with 50 µl of the 2012/13 formulation of Afluria®, a trivalent inactivated influenza vaccine and boosted 4, 6 and 8 weeks later. Antibody titers against the hemagglutinin (HA) of each strain contained in the vaccine were measured by ELISA. Antibody responses were reflective of the engraftment efficacy at the time of immunization. Analysis of the HA-specific antibody response showed IgM was the predominant isotype present, indicating that isotype switching is impaired. Hemagglutination inhibition and neuraminidase inhibition assays showed antibody

responses induced in BLT-humanized-mice are weak, with poor functional antibody titers. Flow cytometric analysis of splenocytes from individual mice indicated the presence of B and T cells in expected proportions, however most B cells were categorized as naïve. Analysis of cytokines secreted following conA activation suggested that the T cells from these mice have an immature phenotype. Although humanized mice may be a useful tool to evaluate the immunogenicity of influenza vaccines, our results indicate that further studies are needed to identify and correct deficiencies in this model.

1.11 Study design and analysis for studies in medical countermeasures with extremely small sample sizes

Li, Xianbin; Higgins, Karen. *U.S. Food and Drug Administration.*

Background: Regulatory approval based on the Animal Rule requires experimental efficacy studies in animals. Conducting studies as small as possible is a guiding principle in animal research. How to design studies and analyze data from studies with extremely small sample sizes is important to speed drug development and to minimize the number of animals used and sacrificed in studies. The objective of this research is to select the best statistical methods for analyzing data from studies with extremely small sizes.

Methods: We will discuss the statistical power under different study designs (balanced and unbalanced) and sample sizes. Recognizing the conservativeness of a Fisher's exact test, the performance of selected alternative statistical analysis methods will be investigated in terms of type I error, type II error, coverage of 95% confidence intervals, and width of the 95% confidence intervals.

Results: Statistical power and type I error rates are investigated and compared.

Conclusions: 1. Unbalanced design is fine. Sometimes more power is achieved from an unbalanced design. But would recommend avoiding an extremely unbalanced design, such as 1 or 2 animals; 2. Fisher's exact test is too conservative; 3. Boschloo's method usually provides more statistical power and is less conservative and with type I error less than the nominal level; and 4. Other methods such as Barnard's test can be used.

Biomarkers & Correlates of Protection

2.1 Biomarker Screening from Post-transcriptional Gene Expression Profiles of Bacillus anthracis Sterne Infected Human Primary Cells

Marasa Bernard[1]; Sung Kidon[1]; Han, Tao[2]; Fuscoe, James[2]; Cerniglia, Carl[1]; Khan, Saeed[1]. *Division of Microbiology[1], Division of Systems Biology[2], National Center for Toxicological Research (NCTR), US Food and Drug Administration, Jefferson, AR 72079, USA.*

Background: *Bacillus anthracis* is a gram positive spore-forming bacteria that causes life threatening disease (anthrax). It affects humans and many species of animals and is classified as a bioterrorism

agent. The spores cause three types of anthrax infections with vastly different lethality outcomes namely; cutaneous (<1%), gastrointestinal (25-60%) and pulmonary anthrax (>90%). Critically, the molecular mechanisms by which *B. anthracis* pathogenic factors influence host immunological responses at different sites of infection are not well understood.

Objectives: We hypothesize that exposure to infectious *Bacillus anthracis* spores generates a post-transcriptional gene expression (GE) profile that can serve as biomarkers for developing medical countermeasures against anthrax exposure.

Methodology: The cells were infected with the spores of *B. anthracis Sterne* at a multiplicity of infection of 10. At 1H, 3H, 5H, and 24H post infection (P.I) cells were processed for electron microscopy, and RNA isolation. GE in human epidermal keratinocytes (NHEK), Intestinal myofibroblasts (InMyoFibs) and Small airway epithelial cells (SAECs) were evaluated using Agilent 4x44k whole genome arrays. MicroRNA profiling was performed using the SBI's MirnomeRT system. The data was imported to Partek Genomics Suite and normalized using 75% scaling. One-way ANOVA was used to calculate the significant gene list with cutoff at FDR< 0.05 and fold change > 1.5.

Results: Microarray analysis revealed an up-regulation of 1729, 4981, 1539 genes and down-regulation of 2178, 5558, 698 genes at 24hr P.I for NHEK, InMyoFibs and SAEC cells respectively. Biomarker filtering using Ingenuity showed that there were 36, 489 and 52 unique biomarker genes among NHEK, InMyoFib and SAEC cells respectively compared to 9 common genes and 68 common miRNA biomarkers.

Conclusion: A number of unique biomarkers that are possibly involved in disease progression were found. The results of this study are important in developing requisite counter-terror measures to the exposure of *B. anthracis*.

2.2 A novel index to monitor physiological systems from arterial blood pressure waveform during hemorrhage in anesthetized swine

Adibuzzaman, Mohammad; Kramer, George C.; Galeotti, Loriano; Merrill, Stephen J.; Strauss, David G.; Scully, Christopher G. *U.S. Food and Drug Administration.*

Background: Identifying patients undergoing hemorrhage requiring immediate care is complicated due to hemodynamic compensation mechanisms that stabilize vital signs until a significant amount of blood loss has occurred. Algorithms that can monitor the hemodynamic stability of patients through analysis of recorded signals (e.g. blood pressure, electrocardiogram) could better inform caregivers of a patient's current physiology leading to earlier interventions and improved outcomes. Markov chain analysis is an analytical technique that can be applied to continuous signals to capture information about the physiological systems affecting the signal. In this study, we used Markov chain analysis to monitor arterial blood pressure dynamics during hemorrhage.

Methods: Continuous arterial blood pressure recordings were made on anesthetized swine (N=7) during a 5 min baseline and a slow hemorrhage (10 ml/kg over 30 min). Empirical Markov chain analysis was applied to 20 sec arterial blood pressure waveform segments. The mixing rate, or the second largest eigenvalue of the transition probability matrix, was calculated for each waveform segment. Due to

compensation mechanisms the blood pressure may remain constant, but the changes in the physiological system maintaining the blood pressure may be captured by the mixing rate.

Results: A change in the mixing rate from baseline estimates was identified during hemorrhage for each animal (median time of 13 min, ~10% estimated blood volume, range of 2 to -33 min). The mixing rate was inversely correlated with shock index, the ratio between heart rate and systolic blood pressure used as a hemorrhage marker, for all 7 animals (median correlation coefficient of -0.95).

Conclusion: The Markov chain mixing rate of arterial blood pressure recordings is a novel biomarker for potentially monitoring and understanding physiological systems during hemorrhage. Monitoring the observable dynamics within physiological signals informs on how the body's control systems are operating to provide a window into a patient's health.

2.3 Identifying potential translational biomarkers to monitor influenza A virus disease progression in an animal model

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Influenza viruses are major causes of respiratory illness in humans and could be used as a bioweapon to trigger a global pandemic. Translational biomarkers that bridge between animal and human trials would be invaluable in confirming the efficacy of new medical countermeasure agents approved under the Animal Rule and determining the optimal dosing paradigms under actual use conditions. The goal of this project was to determine the relationship between various disease biomarkers and the plasma concentrations of known countermeasures (such as Tamiflu) to identify those biomarkers that track the onset and progression of influenza A virus infection and its response to treatment. Female Balb/c mice were infected with mouse-adapted influenza A virus, A/Puerto Rico/8/34 (PR8) (H1N1). Prophylactic (2h pre-infection) and therapeutic (24h post-infection) treatments with either oseltamivir phosphate (OP) or with water (vehicle control) were administered orally for 5 days. The time course and severity of disease were monitored for 10 days post-infection using measurements of body temperature, body weight, lung weight, viral load, mortality and changes in plasma markers. Cytokines (IL-1β, IL-10, IL-12p70, IL-6, KC and IFN-γ) and several candidate miRNAs were profiled as potential disease markers. In the prophylactic group, virus titer significantly decreased from 1 to 2 log ($p<0.05$) during the initial three days of infection compared to controls but increased after treatment was stopped; mortality in the prophylactic group was delayed by 3 days. No significant difference from control was observed in the therapeutic treatment groups, despite a 1 day delay in mortality, suggesting that OP might be more effective when given prophylactically. The expression of IL-6 and KC-GRO cytokines appeared to track non-linearly with viral load but differences among treatment groups were not statistically significant. Among the 48 miRNAs profiled in the prophylactic treatment groups, mmu-miR-494 and mmu-miR-1897-5p ($p<0.01$, CT mean > 3 fold) were identified as potential biomarkers meriting further evaluation. Additional studies are needed to confirm these findings with other strains of influenza.

2.4 Modeling the relationship between hemagglutination inhibition (HI) titer and protection against influenza

Huang, Yin; Anderson, Steven; Yang, Hong. *U.S. Food and Drug Administration.*

Background: The objective of this research is to evaluate the relationship between HI titer in the host and the protection against influenza using modeling approaches. We expect that the results of this research will provide us an insight on whether HI titer is a good predictor of protection against influenza; and if it is, what the level of HI titer needed for a sufficient protection is.

Methods: We first searched available data from human challenge studies that reported post-vaccination HI titer, challenge dose, and post-challenge influenza infection. We grouped the volunteers based on their HI titer levels. We assumed the relationship between challenge dose and infection rate (response) could be described by a beta-Poisson dose-response models that has been used for influenza virus. We estimated the model parameters for each HI titer group, and examined the dependency between host susceptibility represented by model parameters and HI titer. The dose-response model was further modified by incorporating such dependency and fit to the data set.

Results: An exponential dependency between the model parameters and HI titer was identified. We incorporated this into the beta-Poisson dose-response model and fit it with all the available data sets. The parameters were estimated based on each data set and a range of the possible values were obtained. Our model estimates that, during a normal outbreak, the infection rate in individuals with HI titer of 40 or higher will be at least 50% less than that in people with HI titer of 5 or less, although it may not be true for exposure to extremely high doses.

Conclusion: The modified models can be potentially used to identify the critical level of post-vaccination HI titer required for sufficient protection against influenza; and therefore, enhance our ability to evaluate the efficacy and protection offered by future candidate influenza vaccines.

2.5 Pandemic influenza vaccines: New tools to explore adjuvant impact on immune responses and cross-clade protection for Human, Swine and Avian Influenza.

Khurana Surender; Coyle Elizabeth; Manischewitz, Jody; King, Lisa R.; Golding, Hana. *U.S. Food and Drug Administration.*

Background: Pandemic influenza is a persistent threat to global health. The continuing evolution of avian and swine strains contribute to unpredictability and difficulties in preparing vaccines against the next highly transmissible virus. However, efforts are under way to improve vaccine effectiveness against heterologous strains through new vaccine approaches including novel adjuvants, prime-boost protocols, DNA and recombinant virus-like particle (VLP) platforms.

Methods: We have developed novel technologies to delineate the immune correlates of protection during natural infection and after vaccination. Whole Genome Fragment Phage Display Libraries (GFPDL) for pandemic H1N1-2009, H5N1 (Vietnam and Indonesia), H7N7 and H7N9 strains were used to elucidate the complete antibody epitope repertoire following influenza vaccination with different vaccine modalities including: Oil-in-water adjuvanted subunit vaccines (MF59, AS03); heterologous

prime boost approaches (DNA-H5 prime (IM) -> subunit H5N1, LAIV H5N1 (intranasal)-> subunit; Adenovirus4-H5-Vietnam (oral) -> subunit. In addition, Surface Plasmon Resonance (SPR) was employed to investigate the magnitude and antibody affinity of human polyclonal antibody response against different antigenic domains within hemagglutinin.

Results: GFPDL analyses of human sera post-vaccination with oil-in-water adjuvanted H5N1 subunit vaccines revealed more diverse antibody epitope repertoires, targeting large conformational epitopes in HA1 receptor binding domain, compared with unadjuvanted subunit H5N1 vaccination. SPR analyses revealed that oil-in-water adjuvanted vaccines and the prime-boost approaches significantly enhanced antibody affinity maturation to H5N1-HA1 domain (but not HA2) as demonstrated by slower antigen-antibody complex dissociation rates. Importantly, strong correlation was found between increased antibody affinity and the neutralization titers against both homologous (vaccine) and heterologous pandemic influenza strains.

Conclusions: In summary, GFPDL and SPR identified attributes of influenza-specific antibodies, which are important for increased breadth of cross-protection against heterosubtypic influenza strains with pandemic potential. These technologies will help in deciphering the quality of immune responses against future vaccine candidates and contribute to the design of more effective vaccines modalities and novel adjuvants.

2.6 Development and Validation of Electroencephalographic (EEG) Biomarkers for Brain Injury Monitoring

Ye, Meijun; Fisher, Jonathan; Nabili, Marjan; Huang, Stanley; Kim, Yeonho; Liu, Yunbo; Krauthamer, Victor; Civillico, Eugene; Myers, Matthew; Welle, Cristin. *Center for Devices and Radiological Health, U.S. Food and Drug Administration.*

Background: Mild traumatic brain injury (mTBI) is a significant challenge to civilian and military medicine. The development of medical products for the diagnosis and treatment of TBI is impeded by gaps in regulatory science, including a lack of animal models for CNS-specific mTBI and quantifiable biomarkers for device efficacy. We are addressing these gaps by developing a novel murine model of mTBI, and working to identify electrophysiological biomarkers of mTBI using novel flexible electronic diagnostic devices.

Methods: Our controllable high intensity focused ultrasound (HIFU) TBI model mimics the damage created by a blast. The model is evaluated by immunohistochemical and behavioral methods. Electrophysiology biomarkers, including spontaneous brain activity of freely moving mouse and median-nerve sensory evoked potentials (MN-ERP) in the anesthetized mouse, are identified using epidural micro electrocorticography (μECoG) and epidermal EEG (contributed by T. Coleman) technologies. The frequency content of EEG/ECoG signals before and after HIFU-induced TBI are quantified by multitaper FFT (Chronux toolkit and custom Matlab code).

Results: Preliminary work with the HIFU model shows multiple structural and molecular markers of mTBI, including leakage of the blood brain barrier and increased astrocyte and microglial activity. In parallel electrophysiological experiments, novel μECoG recordings show reduced low frequency brain oscillations and a generic reduction in MN-ERP in HIFU-TBI models compared with non-TBI conditions.

Conclusions: Preliminary results indicate the potential of quantitative EEG as a brain injury biomarker, and the possibility for rapid brain injury detection using portable technology. The utilization of novel EEG biomarker detection of TBI will enable future development of portable, field-deployable TBI diagnostic devices for use in a mass casualty situation.

2.7 Vascular re-mapping around a chronically implanted microelectrode in mice

Lozzi, Andrea; Abliz, Erkinay; Greenbaum, Noah; Agrawal, Anant; Krauthamer, Victor; Hammer, Daniel X.; Welle, Cristin. *Center for Devices and Radiological Health, U.S. Food and Drug Administration.*

Background: Implanted recording microelectrodes for brain computer interfaces (BCIs) exhibit long-term degradation of neural signals that limit their performance lifetime. One open question is whether neural or vascular response of tissue around the electrode can be used as an early indicator or biomarker of failure. The objective of this study was to demonstrate optical coherence microscopy (OCM) and two-photon microscopy (TPM) capabilities for neurovascular imaging.

Methods: We performed TPM and OCM on the motor cortex of transgenic mice with sparsely labeled neurons as well as wild type control mice. A silicon-based microelectrode array (Neuronexus, Inc.) was inserted into the transgenic mice. A 2x2 mm area of the mouse skull was removed and replaced with a glass coverslip to enable imaging of the cortex. TPM allowed visualization of the arborization of neural processes, and OCM was used primarily to image the vasculature structure. Each mouse was imaged at least once per week up to 24 weeks.

Results: We observed dilation and neovascularization in the superficial region immediately underneath the window (0-100 μm), presumably from the window surgery. The dilation, which can expand vessels >50% of their original diameter, peaks about day 3 but constricts and returns to original diameter soon thereafter. The new superficial vessel growth peaks on day ~8-10 and stabilizes thereafter. No significant re-mapping of the vasculature in the deeper regions around the electrode was observed, perhaps because only a very narrow tissue region around the electrode is affected. Neural imaging with TPM was not yet performed over a long enough period of time to make observations on longitudinal neural re-mapping.

Conclusions: TPM and OCM are valuable tools that permit investigation of a long-term tissue response associated with implanted electrodes. These imaging modalities may also foster development of new biomarkers for neurotrauma and other medical countermeasure applications.

2.8 Evaluation of Factors Affecting Filovirus Entry into Primary Human Macrophages

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Background**:** In general, filoviruses cause severe hemorrhagic fever in humans with a very high mortality rate. Macrophages play a critical role during filovirus infection by being one of the major sites of virus replication, as well as a source of multiple cytokines. However, the precise role of these cytokines in filovirus pathogenesis is not adequately clarified.

Methods**:** To avoid restrictions related to working under biosafety level 4 (BSL4) conditions, we have optimized the use of filovirus envelope glycoprotein (GP) pseudotyped virus particles, containing either β-lactamase (BlaM) or green fluorescent protein (GFP), in primary human macrophage cultures.

Results**:** Using this model, we have assessed the effects of specific cytokines on filovirus-cell fusion. We have found that pre-incubation of primary human monocyte-derived macrophages (MDM) with interleukin-10 (IL-10) significantly enhanced filovirus entry, an effect consistently observed in cells derived from ten normal donors. In contrast, fusion of IL-10-treated macrophages with influenza hemagglutinin/neuraminidase pseudotyped virus particles was reduced relative to mock treated cells. We then established that the IL-10 effect on filovirus entry is due to increased virus binding and endocytosis. Intriguingly, earlier studies have reported a correlation between elevated serum IL-10 and increased mortality in filovirus infected patients, although the underlying mechanism has not been established. More recently (Panchal, et al.), it was observed that suppression of circulating IL-10 was associated with increased survival in an Ebola virus animal model due to modulation of NK cell function and/or interferon γ levels.

Conclusion**:** Our studies have identified a novel mechanism that may account for the IL-10-mediated increase in filovirus pathogenicity. In addition, the expertise acquired during these studies will be useful in establishing less hazardous systems for development and evaluation of new filovirus countermeasures.

2.9 Development and validation of a Liquid - Chromatography Tandem Mass Spectrometry Method for the Quantitation of Oseltamivir Phosphate and Oseltamivir Acid using Dried Blood Spots: Implications for Pediatric PK and Medical Countermeasures

Chimalakonda, Krishna; Pang, Eric; Patel, Vikram; Boyne, Michael. *Center for Drug Evaluation and Research, U.S. Food and Drug Administration.*

One of the main challenges in conducting pharmacokinetic (PK) trials in infants is the limited blood volume that can be collected. Because only a single sample from an individual infant is typically drawn, the generated PK dataset lacks robustness. As a result, most current medical countermeasure drugs either lack reliable PK data or have no data in infants. The common practice is to extrapolate infant dosages from adult models; however, this method can often lead to inappropriate dosing because infants have different absorption, metabolism, and excretion rates. The dried blood spot (DBS) sampling techniques in infant studies is attractive because DBS requires much less blood volume, making increased sampling possible. However, there are major concerns with using DBS, including a decrease in analytical sensitivity compared to plasma sampling and an effect of hematocrit (HCT) levels on the results. To assess these concerns, we are studying the suitability of DBS in a model system. We have developed a DBS LC-MS/MS method for quantifying oseltamivir, which requires the analysis of oseltamivir phosphate (OP, the prodrug) and oseltamivir acid (OA, the bioactive metabolite). Assay validation was performed to determine stability, precision, and accuracy. In addition, the effect of HCT was explored. We observed no significant loss (< 15%) in the quantification of analytes on DBS cards at room temperature**.** Accuracy and precision of the assay were determined to be ±10 % and ±13%, respectively, for all the quality control (QC) samples, with lower limits of quantitation of 50 ng/mL and 10 ng/mL for OP and OA, respectively. Changing hematocrit levels did not affect the quantitation of OA,

but caused an underestimation of OP quantitation at lower hematocrit levels. Work is ongoing to understand the source of this variation, if it is compound specific, and the potential impact on the utility of the DBS approach.

Devices

3.1 A Point-of-Care Multiplexed Immunoassay for the Detection of Acute Kidney Injury Biomarkers

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Background: We designed a planar surface fluorescent immunoassay to detect Acute Kidney Injury (AKI) biomarkers that performs as well the gold standard, but with less sample volume and is amenable to a Point-of-Care (POC) device. Detecting biomarkers using this format could allow for more rapid diagnosis of kidney dysfunction, which can result following exposure to biothreat agents that contain endotoxin (gram-negative bacteria).

Methods: Microscope glass slides are functionalized to covalently attach antibodies. The sample, containing the analytes of interest, is exposed to the surface, followed by detection using a fluorescently-labeled dye. Vinyl, polycarbonate, and acrylic templates are fabricated on a laser cutter and assembled on top of the functionalized glass slide in order to facilitate immobilization of antibodies and performing the assay. A POC device that employs total internal reflection and generation of an evanescent wave was created to image the glass slides and obtain quantitative data.

Results: Detection of Chicken IgG and Staphylococcal Enterotoxin B (analytes commonly used in literature) had comparable Limits of Detection (LODs) and dose response curves to the conventional method employing a 96-well plate. Urinary biomarkers, Kidney Injury Marker-1 (KIM-1) and Neutrophil Gelatinase Associated Lipolcalin (NGAL), were spiked into buffer and showed slightly higher LODs using the planar surface method as compared to the 96-well plate assay. However, these LODs are lower than the currently-known cut-off levels above which disease may be present. Use of the POC device for analysis demonstrated that proteins can be detected both spatially and spectrally within a single assay when fluorescent labels called Quantum Dots are applied.

Conclusion: A multiplexed immunoassay was developed to detect AKI biomarkers using smaller sample volume than conventional techniques. An evanescent waveguide detection platform was created for POC use. This system can be applied to detect AKI biomarkers in urine following exposure to biothreat agents.

3.2 Removal of a Surrogate for Botulinum Neurotoxin A from Material Surfaces of Different Roughness and Several Reusable Medical Devices

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Background: Botulinum neurotoxin A (BoNT/A) is on the national select agent registry and can be an extremely potent biotoxin if weaponized, as it blocks neuromuscular transmission by decreasing acetylcholine release. The surfaces of reusable medical devices can be contaminated by natural toxins either accidentally or intentionally. This can be a serious problem if expensive reusable medical devices such as ventilators or anesthesia machines are contaminated. Transfer of BoNT/A from medical device surfaces to the patient or health care provider can occur.

Methods: This study focuses on measuring the removal of the enzymatically active light chain A (LcA) of BoNT/A from reusable medical devices and materials. A labeled SNAP-25 (synaptosomal-associated protein of molecular mass 25 kDa) peptide substrate of the enzymatically active LcA was used to quantify the amount of LcA. Various medical device materials with different surface roughness were tested to evaluate the efficacy of removal of LcA as a function of surface roughness.

Results: Each material had four different surface roughness treatments, varying from 0.081 to 3.916 mm. There was not a statistically significant difference in the removal of LcA from these medical device materials for the different surface roughness tested in this study. The removal of LcA from the five medical device surfaces (one anesthesia machine, two ventilators, and two bed rails) ranged from 36-71%.

Conclusion: If a medical device surface is accidentally or deliberately contaminated, it is important to determine if it can be decontaminated to make it suitable for subsequent use. This study demonstrates that LcA of BoNT/A can be removed from several large reusable medical devices and that surface roughness was not associated with removal efficiency.

3.3 Performance Testing of Fast Read Digital Thermometers

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Background: Body temperature monitoring of humans has been an important tool for diagnosing infections, detecting fever, monitoring thermoregulation functions during surgical procedures, and assessing post-surgery recovery. Inexpensive, off-the-shelf digital thermometers are generally used to measure temperature orally or under the arm. Currently, many such thermometers are available with a “fast read” capability, where they produce temperature readings in 5 to 10 seconds. The purpose of this study was to investigate the accuracy of the fast read thermometers compared to our reference thermometer.

Methods: A total of 301 patients (infants to 18 years old) participate in this study when they visited a local pediatrician's office for a checkup or sick visit. We tested three thermometer brands, 20

thermometers of each brand. We calculated the difference between the reference thermometer temperature and the temperatures measured simultaneously with the off-the-shelf thermometers.

Results: Compared to the reference measurements after 120 seconds (two minutes), the off-the-shelf thermometers routinely deviated from the reference temperature at the site, and those deviations were not consistent. The Brand C thermometers had the greatest deviations of up to 3.7F, while the Brand A thermometers had the lowest deviations; however, they still deviated by up to 1.9F. It is obvious that the tested off-the-shelf thermometers had lower accuracy than the indicated ±0.2˚F.

Conclusion: It is possible that the off-the-shelf thermometers are not giving an actual temperature reading. Instead, they are calculating a temperature based on transient temperature values over the first 5-11 seconds of measurement, using an embedded algorithm. We believe that these differences can be caused by the environment and the physiology of the patient. The temperature calculation in the off-the-shelf thermometers cannot take into account patient-based differences, which lead to different transient temperature responses during the first several seconds. Therefore, the inconsistent deviations are not surprising.

3.4 Efficacy determination of different chemical disinfectants in the removal of vegetative bacteria and spores from reusable medical devices

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Reusable medical device surfaces are vulnerable to contamination by select agent pathogens (SAPs). The CDC classified these organisms as Tier 1 SAPs because of their potential to be used in a bioterrorism attack resulting in mass casualties. We investigated cleaning and disinfection of several SAPs: *Burkholderia mallei, Burkholderia pseudomallei, Yersinia pestis*, and *Bacillus anthracis* spores which are the causative agents of glanders, melioidosis, the bubonic plague, and anthrax, respectively. Healthcare professionals must also be wary of bacteria that commonly cause nosocomial infections such as *Clostridium difficile* and methicillin- resistant *Staphylococcus aureus* (MRSA). *C.difficile* vegetative cells and spores can cause severe diarrhea following the treatment of patients with antibiotics, and MRSA is responsible for infections that are difficult to treat due to antibiotic-resistance. *B. anthracis and C. difficile* are capable of forming spores, which are more long-lived and hardier than vegetative cells. Infected patients have the potential to contaminate reusable medical devices with pathogenic microbes, which then may infect healthcare personnel or subsequent patients using improperly cleaned and disinfected reusable devices. We have used surrogate bacteria for the SAPs *(B. thaliadensis*, *Y. pseudotuberculosis*, and *B. atrophaeus* spores) and two bacteria (*C. sporogenes* and *S. aureus*) that are involved with healthcare associated infections to determine the optimal method to remove the bacteria from medical devices. We evaluated the efficacy of commercially available cleaning/disinfecting wipes with various active ingredients to remove the bacteria from the surfaces of an anesthesia machine, hospital bedrails, pulse oximeters and knobs. We also determined the effects of wipe wetness and the influence of soil, such as sebum, on the device. Anesthesia machines and hospital bedrails, which have comparatively smooth and uncomplicated surfaces, were easier to clean than devices with more complex surfaces: the pulse oximeters and knobs. Generally, wipes containing sodium hypochlorite were most effective at removing and disinfecting bacteria from the devices.

3.5 Medical Device Use-Related Hazards During Chemical, Biological, Radiological, Nuclear or Explosive (CBRNE) Emergency Response

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Background**:** Medical devices are often designed and tested for use in clinical settings, with the intended user population. However, devices are used in austere conditions, during emergency response to stabilize and treat victims before transporting them to pre-clinical/hospital facilities. In this research, investigators conducted a systematic literature review and meta-analyses on the impact of PPE on medical device uses during emergency responses.

Methods**:** This systematic literature search comprises peer-reviewed journals in PubMed, Web of Science and EBSCO Science & Technology databases. Studies were appraised independently by H.W. and J.C. or K.S. for eligibility and inclusion. They included high-fidelity empirical evaluation of emergency clinical procedures with medical devices by healthcare responders and receivers in either CBRN-PPE or MOPP in a simulated pre-clinical or hospital environments.
Results**:** 29 out of 92 articles published between 1984 and 2013 were identified for the review. CBRN-PPE and MOPP use increased the time to completion for emergency procedures by as much as 100% and 12% failure rates at first attempts. PPE affects device user emergency clinical task performance through reduced finger and hand motor skills, impaired vision and hand-eye coordination, tactile sensitivity feedback and team communication. However, task performance improved significantly at the second attempts.

Conclusion**:** CBRNE-PPE and MOPP can have significant negative impact on emergency clinical task performance during emergency response. Medical device design modifications and improvement in PPE can improve speed, safety, and accuracy on medical device use during emergency response. Most importantly, increased training under the expected conditions can have a mitigating effect.

3.6 Performance of pediatric face masks during off-label public health emergencies

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Background**:** Respirators, surgical masks and face masks will form the first line of defense in case of public health emergencies such as pandemics and bioterror attacks by reducing the exposure of bio-pathogens. While adult masks have been thoroughly studied because of their relevance to work-related exposures, pediatric face masks that have primarily been used in hospitals settings have garnered very little attention in off-label scenarios (e.g., bioterror attacks).

Methods**:** An in-house capability of characterizing the performance of masks has recently been developed. The aerosols were generated using atomizers that created micrometer droplets, which subsequently reduced in size by drying. Size distributions were determined using standard aerosol characterization techniques (differential mobility analyzers) in the sub-micron size regime (15 nm to 800

nm). The masks were then challenged with a flow of these aerosols, and the effectiveness of the masks was quantified by measuring the aerosol concentration inside and outside the mask. Using this methodology, the effect of several conditions that may arise in off-label use was studied: neutralized versus un-neutralized aerosols, polydispersed versus monodispersed aerosols, and the effect of different breathing rates.

Results**:** Two adult masks and three pediatric masks were used for this study. It was found that the adult masks provided almost 95 % protection under most conditions, and in the entire size regime of 15 nm to 800 nm. In contrast, pediatric masks provided only about 50 - 90 % protection, depending on the model chosen, the condition of the aerosols, and the flow rates used.

Conclusions**:** Existing pediatric masks only provide limited protection in off-label scenarios compared to adult masks. Thus, children may receive higher doses of bio-pathogens in case of a pandemic or bioterror attack, making them a more susceptible population.

3.7 Improved Heart Beat Detection by Using Multiple Physiological Waveforms

Galeotti, Loriano; Johannesen, Lars; Vicente, Jose; Scully Christopher G.; Strauss David G. *Center for Devices and Radiological Health, U.S. Food and Drug Administration.*

Background: Alarm fatigue is a major issue in hospital care resulting from a high number of patient monitor false alarms that could be reduced by merging physiological information from multiple sensors, minimizing the impact of a single sensor failing. Heart rate, one of the most common measured vital signs and responsible for a significant number of monitoring false alarms, can be observed in many physiological signals. We developed a robust heart beat detection algorithm utilizing multiple physiological waveforms (electrocardiogram ECG, blood pressure BP, photoplethysmogram PPG, electroencephalogram EEG).

Methods: A 100 record (each ~10 minutes) training set annotated with beat locations was used for development. Heart beats are detected on ECG and EEG (where residual ECG may be present) using a non-linear QRS detector (U3). BP and PPG pulses are located using a custom derivative-based peak detector. ECG beat-beat intervals longer than a pre-defined threshold are identified as gaps and filled by the BP, PPG, and EEG detections. The algorithm was applied to an annotated test set. Beats were considered true if within 150 ms of an annotation. Sensitivity (Se) and positive predictive value (PPV) were determined for the algorithm and, for comparison, the ECG detector alone.

Results: Training set PPV was 99.97% for both the algorithm and ECG detector alone, while Se of the algorithm was higher (99.82%) than the ECG detector alone (99.47%). On the 100 record test set we achieved Se of 86.57% and PPV of 95.72% with the algorithm.

Conclusion: We developed a robust heart beat detector that fuses information from ECG, EEG, BP, and PPG signals. Implementing this algorithm in a monitoring device could provide continuous heart beat detection when one or more signals are unavailable due to noise or sensor disconnection, lowering the number of false alarms.

Diagnostics

4.1 Photoacoustic Tomography System for In Vivo Detection of Endogenous and Exogenous MCM Biomarkers

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Background**:** Photoacoustic Tomography (PAT) is an innovative technology which enables deep, non-invasive, structural, functional and molecular imaging in biological tissue, based on laser excitation and acoustic detection. This approach has great potential for improving detection of biomarkers of toxic agent exposure and infectious disease, as well as biomarkers relevant to major health care issues such as breast cancer. We have recently completed construction and validation of a PAT system capable of spectral imaging of optical biomarkers (e.g., hemoglobin, nanorods) at depths of several centimeters.

Methods**:** Our custom PAT system combines a tunable near-infrared laser emitting nanosecond-scale pulses with a research-grade ultrasound system. Computer-based system control and data processing algorithms were developed to perform tasks including high-time-resolution triggering and extraction of spectral content from imaging data. Tissue-simulating phantoms with realistic acoustic and optical properties containing optical biomarker solutions in fluid channels were used to characterize PAT system performance.

Results**:** Images showed higher signal at the channel walls than the lumen due to acoustic wave interference within the channel. Contrast increased with radiant exposure and dye concentration, but with diminishing returns at radiant exposures over 40 mJ/cm2. For a target with equivalent absorption to that of human blood and at maximum permissible laser exposure, a contrast ratio of 8.9 ± 0.4 was achieved. Spatial measurement accuracy was shown to be ~88%. Detection of the spectral signature of a contrast agent capable of molecule-specific labeling was demonstrated.

Conclusion**:** We have developed and validated a high-performance PAT system capable of deep spectral imaging in biological tissue. Ongoing investigations with this system include evaluating MCM-relevant biomarker detectability and measurement accuracy, elucidation of safety issues, and development of phantom-based test methods for cancer imaging devices. This system represents cutting edge research infrastructure for CDRH with the potential to impact a wide range of regulatory science topics.

4.2 Development of a Chikungunya virus RNA Reference Reagent for standardization of nucleic acid tests

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Background**:** Chikungunya virus (CHIKV) is an Alphavirus transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, the same vectors that transmit Dengue viruses (DENV), a group of four

Flaviviruses that often co-circulate with CHIKV in the same geographical area. CHIKV has caused explosive epidemics in Africa, Asia and Indian and Pacific Ocean islands. It recently appeared in the Caribbean islands, posing risk to the rest of the Americas. Some CHIKV human infections are asymptomatic. Most cause a febrile illness similar to that of DENV, characterized by high fever, polyarthralgia, headache, back pain, myalgia, nausea, vomiting, and rash. There are no vaccines or specific treatments for DENV or CHIKV. However, DENV infections require prompt differential diagnosis and not infrequently hospitalization to prevent fatalities. Laboratory diagnosis for these viruses is made by serology, viral isolation or by nucleic acid test (NAT), the most sensitive method. There are no FDA-approved CHIKV diagnostic or blood screening assays. The lack of a reference reagent for CHIKV is a barrier for proper evaluation of NAT assays. This work aims to produce a well-characterized CHIKV RNA Reference Reagent (CHIKV-RR) for use as a standard for evaluation of performance of existing assays, and to facilitate the development of NAT assays that fulfill the requirements for blood screening.

Methods**:** The CHIKV-RR candidate was produced by (a) expansion of a CHIKV clinical strain in Vero cells; (b) heat-inactivation (HI) of the viral stock; (c) preliminary in-house titration of the HI CHIKV stock; (d) validation of results in external collaborative studies; (e) formulation of the CBER/FDA CHIKV-RR.

Results**:** Preliminary results showed that the CHIKV stock has a concentration of ~106 PCR-detectable units/ml.
Conclusions**:** The final formulation of the CHIKV-RR was shipped to collaborators for a second round of testing. External results are expected within 3 months, when will be subjected to statistical analysis for assignment of a final number of units.

4.3 A rapid, multiplexed, mobile phone-enabled point of care diagnostic device to detect Category A bioterror agents

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Background: Medical countermeasure surveillance and reporting during and after a public health emergency event requires sensitive and specific detection/diagnostic methods and devices. We are designing, building, and testing a rapid, multiplexed, mobile phone-enabled diagnostic device to detect dengue virus and Ebola virus, Category A bioterror agents, in the field. The goal is to deliver a device that will permit screening for multiple pathogen markers without the need for refrigeration, specialized training, specialized equipment or chemicals. Mobile phone technology is used to analyze the lateral flow data, quantify the results, and upload the results for real-time epidemiology.

Methods: The device is based on lateral flow chromatography, an established technology. Current multiplexing permits assay for up to eight pathogen markers concurrently using one hundred microliters of sample. Monoclonal antibodies have been screened using flow cytometry and lateral flow chromatography to define functional pairs when conjugated to gold nanoparticles and bound to

nitrocellulose paper. Nanoparticle surface chemistries are being evaluated to identify low cost approaches to prepare conjugated nanoparticles. A mobile phone app has been coded to record the image of the multiplexed diagnostic, correct the image for user photography errors, quantify the signal intensities, and upload data to a server, with GIS.

Results: A prototype device that detects and distinguishes the four serotypes of dengue virus, dengue IgG/IgM, Ebola glycoprotein, and ST2 protein has been built and tested. Initial specificity and sensitivity tests using laboratory proteins and human patient serum samples are favorable. The phone app records the data, measures signal intensities, and uploads data for real time epidemiology.

Conclusion: A multiplexed rapid lateral flow diagnostic for field use detects Category A pathogens and uploads data for real-time epidemiology.

New Technologies

5.1 Imaging Enterobacteriaceae Infection with Fluorodeoxysorbitol Positron Emission Tomography

Weinstein, Edward; Ordonez, Alvaro; DeMarco, Vincent; Murawski, Allison; Pokkali, Supriya; MacDonald, Elizabeth; Klunk, Mariah; Mease, Ronnie; Pomper, Martin; Jain, Sanjay. *Center for Drug Evaluation and Research, U.S. Food and Drug Administration.*

Background**:** The Enterobacteriaceae include the biodefense category A pathogen *Yersinia pestis,* as well as *Escherichia coli*, *Klebsiella pneumonia, Shigella* and *Salmonella* species. New tools are urgently needed to identify, quantify, and localize gram negative infections, and to monitor antimicrobial efficacy against drug resistant strains in real time. We describe our development of [2-^{18}F]-Fluorodeoxysorbitol (^{18}F-FDS) as a positron emission tomography (PET) imaging probe for enterobacteria in a mouse model of infection.

Methods**:** ^{18}F-FDS was generated from commercially available [2-^{18}F]-Fluorodeoxyglucose by chemical reduction in less than 30 minutes. Immunosuppressed CBA/J mice developed myositis due to infection with drug-susceptible *E. coli*, extended-spectrum beta-lactamase (ESBL)-producing *E. coli,* or control heat killed bacteria. Ceftriaxone was administered subcutaneously for 24 hours. Animals were imaged before and after antimicrobial treatment by injecting 7.4 Mbq ^{18}F-FDS into the tail vein. CT scans were performed for coregistration with PET images. Tissues were collected for colony forming units (CFU) enumeration, histopathology, and direct gamma counting.

Results**:** ^{18}F-FDS-PET differentiated true infection from sterile inflammation with a limit of detection of 6.2 ± 0.2 $\log_{10}$ CFU *E. coli*. ^{18}F-FDS-PET signal correlated with the bacterial CFU burden. PET signal disappeared following successful treatment against drug-susceptible *E. coli* ($P < 0.01$), but persisted in mice infected with the drug-resistant *E. coli*. An increase in CFU corresponded with disease progression to sepsis and death.

Conclusion**:** ^{18}F-FDS-PET rapidly and specifically localized infections due to Enterobacteriaceae, providing a three-dimensional holistic view within the animal. The efficacy of antimicrobial treatment was monitored by ^{18}F-FDS-PET, demonstrating a PET signal proportionate to the bacterial burden. Therapeutic failures associated with multidrug-resistant, ESBL-producing *E. coli* infections were detected in real-time. Together, these data show that ^{18}F-FDS is an excellent candidate imaging probe for translation to human use. The technique could provide rapid diagnosis and be used to guide antimicrobial therapy.

5.2 Assessment by Ames Test and Comet Assay of Toxicity Potential of Polymer used to Develop a Field Capable Rapid Detection Device to Analyze Environmental Samples

Hebert, Amanda; Gleason, Karen; Battacharrya, Dhiman; Torosian, Stephen*. U.S. Food and Drug Administration.*

In this study, collaborators from FDA/WEAC, MIT and DoD are creating a real time biosensor. Many strategies exist for creation of biosensors, providing the researcher, in this case electrochemically conductive coating options. New and nano-technologies require an examination of their unintended effects due to unique characteristics or processes involved in their creation. In this study the materials used to create the biosensor are assessed for potential cytotoxicity prior to inclusion in the platform. The device is comprised of melt-spun polypropylene which has been coated with Polymerized 3,4-Ethylenedioxythiophene and 3-Thiopheneethanol (P(EDOT-co-3TE)). Copolymer was deposited on the surface of microfibers via oxidative Chemical Vapor Deposition (oCVD) creating a conductive conformal layer 100-300nm thick. Previous studies of electrodeposited PEDOT discovered no change in viability of cells exposed to copolymer however, they did not examine mutagenicity. The Ames Test and Comet Assay were used to examine the mutagenic and cytotoxic potential of the copolymer. The Ames test, an accepted means of assessing mutagenic potential, exposes histidine mutant bacteria to chemicals, scoring reversion rate compared to controls. The Comet assay employs cell lines as targets of chemical exposure. Seven cell lines of varied speciation and organ of origin were examined. Post exposure the contents of treated cells as well as controls are electrophoresed. Development of a comet-shaped tail, due to large numbers of DNA breaks in the genome, indicates higher probability of toxicity. Employing the Comet Assays showed cytotoxicity potential for both monomers but not the copolymer. Similarly, the Ames Test results indicate the copolymer is non-mutagenic. A literature search indicates this is the first “bottom up” approach to biosensor development. The results of this study demonstrate the safety of a PEDOT based biosensor and highlight FDA FSMA goals of employing novel technologies as well as stewardship and responsible implementation of better public protection.

5.3 Novel Fiber-Optic Fourier Transform Infrared (FO-FTIR) Spectroscopy Platforms for Label-Free Remote Sensing of Biochemical Contamination

Hassan, Moinuddin; Ilev, Ilko*. Center for Devices and Radiological Health, U.S. Food and Drug Administration.*

Background: Medical device contamination has become a critical and prevalent public health concern as devices are being extensively used in clinical practices. In order to prevent transmission of infection to patients and healthcare personnel caused by device contamination, the development and implementation of novel test methods for quantitative, accurate, easy-to-use, and real-time detection of

contaminations is required. Conventional clinical *off-site* methods based on swab/wipe sampling are not sensitive enough and can be time consuming.

Methods: We have developed an alternative fiber-optic infrared signature based sensing platforms integrating reflectance, transmission and Grazing Incident Angle (GIA) approaches to the fiber-optic Fourier transform infrared (FO-FTIR) spectroscopy sensing methodology for label-free, remote, and real-time detection of contaminations on medical device surfaces.

Results: We demonstrated the sensitivity of the FO-FTIR reflection and GIA measurement modality for non-contact, label-free identification of endotoxin as well as protein samples such as bovine serum albumin (BSA). The developed system can identify ≤ 0.0025% (≤ 4 x10^{11} molecules) protein (BSA) and 0.5% (0.5 EU/ml) endotoxin concentration. In addition, we also investigated sensitivity of the FO-FTIR as compare to colony forming units of different types of bacteria such as *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas.*

Conclusion: The novel FO-FTIR sensing platforms combined with signal recognition statistical methods has a potential for non-contact, real-time, in-situ detection and identification of biochemical toxins such as botulinum toxin, ricin, or aflatoxin within the FDA and CDC recognized threshold limits.

5.4 Identification of a Novel Rhabdovirus in the Sf9 Insect Cell Line Using Degenerate PCR and Next Generation Sequencing

Ma, Hailun; Galvin, Teresa, A.; Glasner, Dustin, R; Shaheduzzaman, Syed; Khan, Arifa, S. *Center for Biologics Evaluation and Research, U.S. Food and Drug Administration.*

Background**:** Sf9 cell line, derived from *Spodoptera frugiperda*, is used as a cell substrate for the development of various investigational biological products and the manufacture of two U.S. licensed viral vaccines. Extensive testing has not previously identified any viruses in this cell line.

Methods**:** Sf9 cell line was obtained from ATCC (CRL-1711) and grown as an adherent culture in Grace's Supplemented Insect Medium supplemented with 10% fetal bovine serum. Total RNA was prepared from Sf9 cells and particle-associated RNA was obtained from cell-free supernatant for analysis using degenerate PCR assays, transmission electron microscopy, infectivity assays, and 454-next generation sequencing with extensive bioinformatics analysis.

Results**:** A novel Sf-rhabdovirus was identified in Sf9 cells. Sequence analysis of the assembled virus genome showed the presence of five ORFs corresponding to the N, P, M, G, and L genes. The Sf-rhabdovirus was related in a limited region of the L protein gene to Taastrup virus, a newly discovered member of *Mononegavirales* from a leafhopper (Hemiptera), and also to plant rhabdoviruses, particularly in the genus *Cytorhabdovirus*. Sf-rhabdovirus morphology was confirmed by transmission electron microscopy of filtered supernatant samples from Sf9 cells. Infectivity studies indicated potential transient infection by Sf-rhabdovirus in other insect cell lines, but there was no evidence of entry or virus replication in human cell lines.

Conclusion**:** The results indicate that next generation sequencing with extensive bioinformatics analysis may further enhance cell substrate safety by complementing currently recommended conventional virus detection methods for identification of novel viruses.

5.5 Performance Characteristics and Biodefense Applications of Next Generation Sequencing

Young, Megan; Li, Zhihua; Colatsky, Thomas; Wood, Steven. *Center for Devices and Radiological Health, U.S. Food and Drug Administration.*

Background: Viral hemorrhagic fevers, such as Ebola and Marburg, are classified by the CDC as Category A Bioterrorism Agents/Diseases. As new strains/species emerge, they are generally sequenced to determine a genetic basis to any altered pathology seen. It took ten days to sequence 70% of the 2007 Bundibugyo Ebola epidemic in Uganda. Next generation sequencing is a faster, more cost effective means of sequencing we think is uniquely suited to the rapid identification of biothreat agents. We intend to determine the suitability of Ion Torrent for this application by establishing performance characteristics, such as sensitivity, specificity, detection limits, and reproducibility.

Methods: Viral proteins GP and VP35 play a large role in virulence in Ebola and Marburg viruses. Plasmids containing the sequence of these viral proteins have been sequenced by Illumina sequencing. We sequenced these plasmids using Ion Torrent and compared the results to the Illumina sequencing. Alignment of sequences was performed using TMAP (IonTorrent) and bowtie2 (Illumina), respectively. Coverage was determined using R-based in-house scripts and Rsamtools. Variances were called using samtools.

Results: Ebola and Marburg GP have both been sequenced by Illumina and Ion Torrent, resulting in 100% identical sequences, with markedly less average coverage by the Ion Torrent than Illumina. We recently received the Illumina sequencing for Ebola and Marburg VP35 and will perform a similar comparison for the VP35 sequencing done on Illumina and Ion Torrent.

Conclusions: These studies indicate Ion Torrent may be a viable means of rapid sequencing. With less average coverage and in only four days, we generated sequencing results identical to Illumina for Ebola and Marburg GP. These viruses have small genomes and one Ion Torrent run would likely yield sufficient coverage to identify single nucleotide differences between emerging strains/species and previously known strains/species. We plan to extend this analysis to samples containing viral RNA.

5.6 Cytobank: Enabling Single-Cell Analysis and Personalized Medicine

Kotecha, Nikesh; Irish, Jonathan; Krutzik, Peter. *Cytobank.*

Background: The promise of next-generation therapies, drugs and diagnostics rely on the ability to selectively target pathways and cell subsets - increased selectivity can mean the difference between a broad therapy with significant side effects and a focused therapy that selectively targets diseased cells. Single-cell technologies such as flow and mass cytometry are positioned to fulfill this promise but have been hampered by the analytics.

Methods: We created Cytobank, a comprehensive Web-based tool for analyzing single-cell data. Users upload their data, annotate experimental conditions, and then can produce a variety of visualizations and analyses. The platform also includes advanced analysis tools such as SPADE, for clustering single cells, and tSNE, for analyzing nonlinear relationships between different parameters. Critically, Cytobank is uniquely suited to handling large studies, such as longitudinal clinical trials or animal model studies.

Results: Cytobank has been adopted by many pharmaceutical companies, universities and private research organizations. The platform has also been used as an open-access Web resource for other researchers to critically review published data.

Conclusion: Platforms such as Cytobank are overcoming the challenges associated with large single-cell datasets and helping users understand biological systems controlling development and cell-cell interactions, thus enabling precise targeting of abnormal signaling to specifically kill or modulate diseased cells.

Surveillance

6.1 Association rule mining in the U.S. Vaccine Adverse Event Reporting System

Wei, Lai; Scott, John. *U.S. Food and Drug Administration.*

Spontaneous adverse event reporting systems are critical tools for monitoring the safety of licensed medical products. We apply association rule mining (ARM) in the U.S. Vaccine Adverse Event Reporting System (VAERS) for the detection of potential vaccine-syndrome associations. ARM finds associations between one vaccine and one or more adverse events (i.e., a syndrome). This is in contrast to currently used signal detection algorithms, which are designed to find bivariate associations between a vaccine and a single adverse event. By detecting vaccine-syndrome associations in addition to vaccine-adverse event associations, our approach is sensitive to potentially complex signals, which may be particularly important when monitoring novel medical countermeasures products, such as pandemic influenza vaccines. We find that ARM can detect a variety of potential vaccine-syndrome signals efficiently. To reduce the number of redundant association rules found based on our association rule mining algorithm, we propose a method for post-processing of association rules so that they can be used as a screening method to identify patterns that may need further investigation in the vaccine safety surveillance.

6.2 Logistic Regression Likelihood Ratio Test Analysis with Harnessing Graphics to Explore Safety Data in the Vaccine Adverse Event Report System (VAERS)

Nam, Kijoeng; Russek-Cohen, Estelle. *Center for Biologics Evaluation and Research, U.S. Food and Drug Administration.*

Passive surveillance of vaccine safety data presents several challenges. Vaccines differ from drugs in that they are often given to healthy individuals according to a standardized schedule while drugs treat

established illnesses. Vaccine exposure is difficult to estimate since the number of doses administered is not usually known. Empirical Bayes (DuMouchel; 1999) and LRT approaches (Huang et al.; 2011) provide ways of assessing many vaccine-adverse event combinations in this setting. In conjunction with traditional surveillance, these newer tools can identify vaccine-event combinations that might warrant further exploration as potential safety signals. Patterns of adverse events may vary by demographic group (e.g., congenital disorders that are identified in the first year of life). We have developed a novel approach which combines logistic regression and LRT to allow for adjustment to potential confounders and effect modifiers, such as age, gender, and concomitant vaccines. We present several graphical approaches for examining VAERS data to demonstrate these differences and to aid medical reviewers in assessing and prioritizing vaccine adverse events for further investigation.

Other

7.1 PBPK Models of Renally Eliminated Drugs and Their Application in Evaluating the Effect of Patient Factors

Hsu, Vicky; Vieira, Manuela dLT; Zhao, Ping; Zhang, Lei; Zheng, Jenny; Nordmark, Anna; Gil Berglund, Eva; Giacomini, Kathleen; Huang, Shiew-Mei. *U.S. Food and Drug Administration.*

Background: Renal clearance of drugs may significantly be affected by patient factors, such as renal impairment (RI) and/or drug-drug interactions (DDI). The objective of this study is to develop physiologically-based pharmacokinetic (PBPK) models, using a mechanistic kidney framework, to predict the effect of RI and DDI on the pharmacokinetics of three renally-cleared drugs.

Methods: Three substrate drugs - oseltamivir carboxylate, cidofovir and cefuroxime - were selected because they are all mainly renally cleared with active secretion. Additionally sufficient in vivo data existed to define transporter-mediated clearances for a net basolateral uptake transporter (based on plasma data) and for a net apical efflux transporter (based on urine data). PBPK models were developed using Simcyp® (v12.1). For RI, a system-dependent parameter termed PTCPGK (proximal tubular cells/g kidney) was modulated to explore its application in predicting drug exposure changes in severe renal impairment. For DDI, probenecid was selected as the perpetrator drug to competitively inhibit the net basolateral uptake transporter defined in the substrate models. Probenecid Ki values were evaluated to practically inform its application in predicting drug exposure changes in DDI involving each substrate drug.

Results: The results showed that one can use PBPK approaches to predict the effect of severe renal impairment on the exposure change of drug, assuming a 10-fold reduction in functional tubular cells with reduced filtration rate in the model. Additionally, one can also predict the effect of inhibition on kidney uptake transport by probenecid using a conservative in vivo Ki (≤1 µM).

Conclusion: This study demonstrated the practical use of PBPK modeling to evaluate RI and DDI on kidney transporters.

7.2 Mechanisms of Neuraminidase Inhibitor Transport across the Blood-Brain Barrier

Lin, Lawrence*; Chen, Eugene*; Hsu, Vicky; Zhao, Ping; Zhang, Lei; Huang, Shiew-Mei; Giacomini, Kathleen (*These authors contributed equally to this work). *University of California, San Francisco and U.S. Food and Drug Administration.*

Background: Pediatric patients with influenza receiving neuraminidase inhibitors may be at increased risk of neuropsychiatric events. Physiochemical properties of most neuraminidase inhibitors predict low membrane permeability, so their passage into the central nervous system (CNS) via the blood-CSF or blood-brain barriers (BBB) indicates possible involvement of membrane transporters. The goal of this study was to determine the mechanisms of transport of neuraminidase inhibitors and other antiviral agents into the CNS by examining their interactions with relevant solute carrier (SLC) transporters expressed in CNS barrier tissues.

Methods: HEK-293 cell lines stably overexpressing transporters found in the human BBB, in particular LAT1 (*SLC7A5*), OAT1 (*SLC22A6*), OAT3 (*SLC22A8*), OCT3 (*SLC22A3*) and MATE1 (*SLC47A1*) were tested for their ability to transport 3H-labelled oseltamivir phosphate, oseltamivir carboxylate (OC), zanamivir, amantadine and cidofovir in both a concentration and time-dependent manner. Results were analyzed with Graphpad Prism 5.

Results: Amantadine was not found to be a substrate nor can it completely inhibit LAT1, OCT3 or MATE1 activity. Cidofovir was found to be a partial inhibitor of OAT1 and was a substrate of OAT3 with a K_m=5.3 µM and V_{max}=0.35 nmol/min/mg protein. Oseltamivir phosphate and zanamivir were not found to be substrates of OAT3 or LAT1. OC was found to be a substrate of OAT3 with a K_m=762 µM and V_{max}=0.65 nmol/min/mg protein.

Conclusions: Cellular uptake studies confirmed that OAT3 transports both cidofovir and OC. These studies suggest a mechanism of cidofovir and OC entry into the brain through OAT3. Further studies are needed to determine whether inhibitors of OAT3 may be used to modulate CNS levels and side effects of cidofovir and OC.

7.3 Expanded Access Protocols to Treat Orthopox Infections and Complications from Smallpox Vaccination in Military Personnel and Department of Defense Health Beneficiaries

Karaszkiewicz, James W.; Devlin, Frances K.; Berecz, Michael J.; and Kishimori, Jennifer M. *Force Health Protection Division, US Army Medical Materiel Development Activity*

Background: DoD policy states that licensed drugs and vaccines will be used whenever they are available. The number of FDA-approved countermeasures for biological warfare agents is limited for a variety of reasons, including the low rate of natural occurrence of many of those diseases. Adequate preparedness requires a mechanism to provide medical response capability even if the products are investigational, while still minimizing risk to patients and ensuring appropriate safety monitoring and control of product.

Methods: DoDI 6200.02 assigns the Secretary of the Army as Lead Component for oversight of the use of unapproved products under IND protocols and Emergency Use Authorizations for Force Health

Protection Programs. Expanded Access to Investigational Drugs (21 CFR 312 Subpart I) provides a mechanism to deliver treatment or prophylaxis, outside the context of a clinical research protocol, to high risk, infected, or exposed individuals using products not yet approved in the US for that indication.

Results: Protocols defining the inclusion/exclusion criteria; treatment procedure; safety monitoring and reporting requirements; regulatory compliance; and roles and responsibilities of investigators (treating physicians) and other protocol staff have been developed for a variety of biothreats in an effort to preserve Force Health Protection and Readiness. We describe here protocols for the treatment of orthopox infections, including smallpox and serious adverse events potentially associated with smallpox vaccination, in DoD personnel and associated individuals. Such protocols are distinct from EUAs with respect to requirements for approval, circumstances under which they can be used, population size, reporting requirements, and duration.

Conclusion: Expanded Access protocols for treatment present a valuable mechanism to deliver needed medical response for low probability/high consequence threat agents. Given the lengthy duration of medical product development, and the difficulty of conducting the studies needed for full approval, these protocols provide an Interim Fielding Capability until full approval/licensure is achieved.

7.4 Imaging Capillary Electrophoresis Discriminates Slow from Fast Asparagine Deamidation that May Predict Potency for Recombinant Protective Antigen Anthrax Vaccines

Powell, Bradford; Bourdage, James; Sun, Robin; Crowe, Sherry; Moore, Sam; Hirst, Karie; and Fusco, Peter. *PharmAthene, Inc.*

Background: Candidate next generation anthrax vaccines employing purified recombinant protective antigen (rPA) are sought to replace the legacy vaccine. rPA structure degrades principally by spontaneous deamidation at several asparagines, yielding isoforms with increased negative charge and other structural rearrangements. Since no single site is responsible for loss of vaccine potency, shelf life relates to deamidation at multiple sites. However, establishing causal association between specific deamidation and potency loss is technically challenging and not practical to measure. Alternatively, charged isoform classes can be quantified by imaging capillary electrophoresis (iCE) and correlated with potency.

Methods**:** iCE and a mouse immunogenicity assay using toxin neutralization to measure relative potency (RP) were assessed for stability indicating properties under forced degradation conditions and long-term storage at 2-8^{o} C of an rPA final drug product (SparVax®). iCE deamidation results were also compared to a fluorescent ELISA for measuring antibody binding to immunogenic domains of rPA and the rabbit aerosol challenge model for efficacy.

Results: Analysis of various iCE peak data showed biphasic trending at 5oC for 2 lots, with rapid deamidation representing most of the degradation (70% - 88%) after 15 months, and slower-rate deamidation comprising the remainder of the 27 months test period; however, there was no statistically significant difference in RP among 21 data points over the first 15 months and no loss of rabbit efficacy at 23 months. Forced degradation studies resulted in greater deamidation (95% at 37^{o}C; 93% at 25^{o}C) before a notable loss in RP.

Conclusion: These results confirm prior reports of at least two major rPA deamidation rate classes, with biological function unaffected by sites of rapid deamidation. Ongoing assessments with iCE metrics can now define trending by slow-rate deamidation as critical attributes for rPA structure and biological activity to build a stability model for forecasting product shelf life.

7.5 Expanded Access Protocol Using Arbekacin to Treat Infections caused by Multidrug-Resistant Organisms in Military Personnel and Department of Defense Health Beneficiaries

Madock, Christa M.; Berecz, Michael J.; Zapor, Michael J.; and Kishimori, Jennifer M. *Force Health Protection Division, US Army Medical Materiel Development Activity.*

Background: DoD policy states that licensed drugs and vaccines will be used whenever they are available. DoDI 6200.02 assigns the Secretary of the Army as Lead Component for oversight of the use of unapproved products under IND protocols and Emergency Use Authorizations for Force Health Protection Programs. The growing prevalence of bacterial antibiotic resistance and the paucity of antibiotics in the pipeline for FDA approval necessitate investigating effective antibiotics approved elsewhere.

Methods: Arbekacin sulfate, an aminoglycoside antibiotic used in Japan for treatment of patients with pneumonia or sepsis caused by methicillin-resistant *Staphylococcus aureus* (MRSA), has a broad spectrum of activity including many multidrug-resistant Gram-negative bacteria, and is resistant to most aminoglycoside modifying enzymes. Arbekacin is not FDA-approved. Expanded Access to Investigational Drugs for Treatment Use (21 CFR 312 Subpart I) provides a mechanism to deliver treatment or prophylaxis to high-risk, infected, or exposed individuals using products not yet approved in the U.S. for that indication outside the context of a clinical research protocol, while still minimizing risk to patients and ensuring appropriate product control and safety monitoring.

Results: Here we describe provision of IND arbekacin via protocol to enable treatment of DoD beneficiaries with infections caused by multidrug-resistant organisms when treatment with other antibiotics cannot be used due to unavailability, intolerance, contraindications, or treatment non-response. This project involves collaboration with Force Health Protection Division, Walter Reed National Military Medical Center, Walter Reed Army Institute for Research, Johns Hopkins Medical Institutions, and Meiji Seika Pharma Co., Ltd. Circulation of recruitment flyers and outreach to VA and other military facilities have been ongoing.

Conclusion: Investigational arbekacin is readily available to treat DoD beneficiaries. This project exemplifies a collaborative approach to provide an investigational treatment to the military community and may impact future treatment strategies against multidrug-resistant infections.

7.6 A stock-and-flow simulation model of the U.S. blood supply in support of planning for emergency preparedness and medical countermeasures

Simonetti, Arianna; Ezzeldin, Hussein; Anderson, Steven; Walderhaug, Mark; Forshee, Richard. *U.S. Food and Drug Administration.*

Background**:** An adequate and resilient blood supply is critical for public health. Decision makers need a tool to assess how the blood supply would be affected by disruptions during emergency and non-emergency situations.

Methods: We developed a stock-and-flow computer simulation model of the U.S. blood supply based on contributed blood collections and utilization data to evaluate the daily availability of blood units in response to a pandemic and a mass-casualty event, independently. The stochastic discrete-event simulation model is able to provide either national or regional quantitative estimates with uncertainty on the daily amount of Red Blood Cell (RBC) units available in the system for the whole supply in aggregate or by ABO/Rh blood type. The model includes a simplified inter-regional blood transfer system to capture specific dynamics of blood demand generated from one region supplementing stocks to other regions.

Results**:** Preliminary results on pandemic show that, on average, the steady-state level of the overall U.S. blood supply reduced by 26% (402,000 RBC units) compared to non-emergency situation. The recovery time to pre-pandemic steady-state levels occurs more than six months after the start of the pandemic. Inter-regional transfers of the most prevalent blood types, A+ and O+, increase by 14% and 83%, respectively. Simulation results on a mass-casualty event demonstrate a significant increase of transfers of blood units to the impacted region. Even so, the model estimated a substantial regional shortfall of 300,000 RBCs units. For this scenario, the most transferred blood types were O-, followed by A+.

Conclusion**:** This model can advance the Medical Countermeasures Initiative by informing essential planning of the potential impacts of any time-sensitive crises (such as pandemics or mass-casualty events) and assisting in the development of sound regulatory policy and strategic plans for emergency preparedness to help mitigate morbidity and mortality associated with insufficient availability of blood.

7.7 Cross-reactivity of licensed oil-in-water adjuvanted H5N1 vaccines

Chada, Kinnera; Lee, Martha; Golding, Hana; Anderson, Steven; Yang, Hong. *Center for Biologics Evaluation and Research, U.S. Food and Drug Administration.*

Background**:** Pandemic influenza is a persistent threat to global health. The continuing evolution of avian and swine strains contribute to unpredictability and difficulties in preparing vaccines against the next highly transmissible virus. However, efforts are under way to improve vaccine effectiveness against heterologous strains through new vaccine approaches including novel adjuvants. The oil-in-water adjuvanted (AS03 or MF59) H5N1 vaccines were found to be highly immunogenic in humans at antigen sparing doses.

Methods**:** In this study, a systematic review of human clinical data was performed to assess the cross-reactivity of immune sera generated by oil-in-water adjuvanted H5N1 influenza vaccine strains

heterologous H5N1 strains that were isolated from poultry to human transmissions in 2004-2005. A meta-analysis was performed to assess the cross-reactivity of clade 1 to clade 2.1 (A/Vietnam/1194/2004 to A/Indonesia/05/2005), clade 1 to clade 2.2 (A/Vietnam/1194/2004 to A/turkey/Turkey/1/05) and clade 2.1 to clade 1 (A/Indonesia/05/2005 to A/Vietnam/1194/2004) strains using data from published studies searched using the COSI (Core-Standard-Ideal) protocol.

Results: The analysis results suggested that oil-in-water adjuvanted H5N1 vaccines induced cross-neutralizing antibodies against H5N1 viruses representative of clade 1 and 2 sub-clades that have caused infections in humans. These licensed oil-in-water adjuvanted H5N1 vaccines appear to exhibit broad cross-clade immunity, a desired feature for an optimal prepandemic stockpiled vaccine that has not yet been demonstrated by unadjuvanted H5N1 vaccines.

Conclusions: These encouraging results shed light on the ability of oil-in-water adjuvanted H5N1 vaccines to induce broad cross-reactive immune responses. Stockpiled H5N1 adjuvanted vaccines may allow population priming in the early pandemic phase, slow down the course of pandemic, and provide time needed for development of an effective strain-specific vaccine.

7.8 The Australian Medical Countermeasures Consortium and Medical Countermeasure Products Australia

Pradera, Felicia; Lester, David; Rayner, Craig; Farrell, Leigh. *Defence Science and Technology Organisation, Australia.*

The Medical Countermeasure (MedCM) Consortium is a four nation partnership involving the Defence and Health Departments of Australia, Canada, the United Kingdom and the United States. The Consortium seeks to develop MedCM including drugs, vaccines and diagnostics to assist with all-hazard preparedness and response. Within Australia, the Departments have actively engaged the biotechnology and medical technology communities to understand the current level of capability and infrastructure for the development of MedCM products against chemical, biological and radiological and pandemic threats and emerging infectious diseases.

The Departments commissioned a report which included a series of surveys and workshops to understanding Australia's capability, both common and unique, which could provide beneficial programs and products to the Consortium. Of particular note is that whilst modest in size, there exists representation of all the key components (people, products and facilities) necessary for Australia to be able to significantly contribute to global MedCM product development activities. During the initiative, a groundswell of enthusiasm was generated and has led to a self-assembly of industry, academic, government and research institute stakeholders to form a national taskforce called Medical Countermeasure Products Australia (MCPA).

MCPA is in its infancy and its initial aim is to: develop a fit-for purpose, sustainable public-private business model to support MedCM product development; demonstrate national capability in developing MedCM products by generating measurable, validated and meaningful short-term MedCM product outcomes; focus on regional threats; and secure long term funding.

NOTES

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